Escape of Introduced Ornamentals in Asteraceae - with Main Focus on *Tagetes patula* L. in Western Ethiopia

by
Agnethe Birkeland Salvesen

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Department of Biology
University of Oslo
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Agnethe Birkeland Salvesen
Blindern, Oslo
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Abstract

Introduced plant species may lead to negative consequences for the local community by outcompeting other species, and in extreme situations decrease biodiversity. It is well known that once an invasive species becomes firmly established, its control might be difficult and eradication may be more or less impossible.

Benshangul Gumuz National Regional State in Western Ethiopia inhabits several endemics, and is not very well studied, so far. Features of *Tagetes patula* and *Zinnia elegans,* two introduced ornamentals that has escaped cultivation in the area, will in this thesis be studied. The endemic species *Bidens prestinaria* will be used in some of the experiments for comparison. People in Western Ethiopia are dependent on their crops. An increasing growth of the already naturalized ornamentals might have negative consequences in this regard.

Successful invasion is associated by characteristics as (among others), a large seed production, a soil seed bank, a rapid growth rate, and a stimulated growth of side shoots after grazing/trampling. *Tagetes patula* possess all these characteristics. *Zinnia elegans* was not found escaped to the same degree as *Tagetes patula*, and was accordingly regarded not to be as potentially invasive as *T. patula*.

A species association analysis revealed that *Tagetes patula* was mainly found in the association characterised by tree species, and the association was also containing species demanding some shade and moisture. It was less frequently found in associations were the species composition represented open and more arid habitat types. *Bidens prestinaria*, however, preferred both associations.

To study if fire could be a way to control the species, diaspores of *T. patula* and *Z. elegans* were exposed to different fire simulation treatments. Surprisingly, the result suggested that diaspores of the two ornamental species, to a certain degree will withstand fire, unless the heat becomes excessive. The near endemic species *Bidens prestinaria* was apparently not affected by the heat treatments.

1. Introduction

1.1 Definitions

Biodiversity is in this paper defined as the variety of living organisms from all sources including terrestrial, marine and other aquatic ecosystems and the ecological complexities of which they are part. This includes diversity within species, between species and of ecosystems (UNEP 1992, NOU 2004).

Species present in community assemblages are generally categorized as being either **endemic -** distribution limited to a certain area, **native/indigenous** – the species is not recently introduced, it has either originated in the area, or is the result of prehistoric immigration, or **introduced/exotic** resulting from historical immigrations including natural range extensions and human-mediated introductions (Myers & Bazely 2003).

All species that are expanding their ranges into new areas are considered to be "**colonizers**", but only those that have large impact on the existing habitat are called "**invaders**" (Myers & Bazely 2003). The most accurate use of the term "**invasive species**" is species introduced from a different area (most often a different continent), which first becomes stabilised, then increase in density, and at last expand rapidly across the new habitat. **Weeds** are defined as plants that can grow at high population densities and may have a negative impact on other plants valued by humans. Many weeds are members of the native flora in an area (Myers & Bazely 2003).

Naturalized species, in this paper, are those that have become established to the point where they apparently behave as native species.

1.2 About introduced plant species in general

The human population is every year increasing, particularly in tropical and subtropical regions. Consequently the pressure on natural resources to feed, house and provide other basic necessities for people also increases. As a consequence new areas are inhabited, and land use is changed to more profitable systems. Plant species have been and are being introduced all over the world. In a world without borders, few if any areas remain sheltered from these immigrations (Mack *et al.* 2000). Import of exotic species, that are known to be useful elsewhere in the world, takes place to an increasing degree (Hailu Shiferaw *et al.* 2004).

Many of the major crop and domestic species that sustain the human population have been introduced species (Pimentel et al. 2000) and have given clear benefits to humans all over the world (Myers & Bazely 2003). Through history e.g. maize was introduced to the Old World from Mexico, rice to Africa from Asia and coffee to Brazil from Ethiopia. Eucalyptus was brought from Australia to Africa, initially to defeat malaria, but is now an important for fuel. Several plant species have been introduced to control soil erosion, particularly plants that have rapid growth and tenacious existence (Figure 1.1).

Figure 1.1 Non-native grasses are being used to prevent the roadsides from eroding under heavy rains. Photo: A.B.S. 29 Sept. 2005 from road between Nekemte and Assosa.

Also ornamentals are intentionally introduced. In for example the USA, gardening has ranked in the top five favourite leisure-time activities in six Harris polls conducted from 1995 to 2001 (Myers & Bazely 2003). This has created a public demand for new and exciting species and international trade in plants and plant seeds (Mack & Lonsdale 2001, Myers & Bazely 2003). The result is that gardeners and horticulturalists frequently and intentionally spread ornamental plant species (Reichard & White 2001).

Introductions of alien species in an area might have unexpected consequences in several respects. A general predictive theory of species invasions is lacking (Peterson & Vieglas 2001). The species which are useful in some areas are not necessarily a success in other areas. Under unfortunate circumstances, species that have given positive contributions somewhere, may escape proper management and become serious invading weeds elsewhere (Hailu Shiferaw et al. 2004).

Figure 1.2 Road construction and soil erosion are disturbances that might promote invasion of exotic species. Photo: A.B.S. 4 Sept. 2005 Along the road to Asossa.

Alien plant species are often favoured by human activities (Diamond 1989), especially disturbances (see Figure 1.2). Therefore, the invasion of alien plant species might generate a biogeographic structure which shows the imprint of the geography of human activities. Stadler *et al*. (2000), however, found that the distributional patterns of alien species corresponds to the patterns of native species, and that the reason for this might be that environmental conditions act as filters generating similar patterns of distribution.

Introductions might have serious consequences for ecological, economic and social systems. Once an invasive species becomes established, its control may be difficult and eradication sometimes impossible (Pimentel *et al.* 2000). Moreover, the impact of invaders on natural communities, i.e. biodiversity and ecosystem processes, can be serious (Chapin III *et al.* 2000, Hailu Shiferaw *et al.* 2004). Exotic weeds in conservation areas are increasingly recognized as representing a major threat to the preservation of biodiversity, and can profoundly alter ecosystem structure and function (Cronk & Fuller 1995). In extreme situations introduced species might (on a global scale) contribute to a biodiversity crisis. On a regional scale the introduced species may outcompete native vegetation and become annoying or devastating weeds in farmlands.

Exotic weeds can become very aggressive in their new habitat if the ecological conditions are favourable (Mack *et al.* 2000). The reason for this is that they often outcompete natural species not adapted to competition. They also lack the "natural enemies", such as insects that feed on the plant and pathogens that cause diseases that kept them in check in their native range (Hailu Shiferaw *et al.* 2004).

The actual invasion of an environment by new species is influenced by three factors (Lonsdale 1999): (1) the number of propagules entering the new environment, i.e. propagule pressure: (2) the characteristics of the new species: (3) the susceptibility of the environment to invasion by new species, i.e. invasibility.

Even though some species are known to present a threat to native vegetation, little is known about the impact of most introduced species. It has for example been proved that in the presence of introduced species, soil biota-bacteria and fungi (Belnap & Phillips 2001) change. Dramatic consequences have been that flood regimes have been altered, and soil properties changed. With the introduction of plants and plant products, associated plant diseases might become widespread. Introduced plant diseases have probably had stronger impacts on native plant communities than have their original plant host. In some cases, introduced diseases have in a major way changed the species composition of forests (Myers & Bazely 2003).

Kendle and Rose (2000) present five important arguments that emphasize that native species should be preferred over introduced species in landscape management: (1) Natives may be hardier than exotics, better adapted to the local environment. (2) Exotics may become invasive and outcompete natives. (3) Native plant species support more associated species such as insect herbivores. (4) Their genetic diversity represents unique adaptations that should be protected from contamination through gene flow from exotic species. (5) Native plants define the landscape character. Some kind of regulation might be necessary to avoid that plant species turn into weeds, but commercial interests might counteract implementation of strict quarantine regulations (Myers & Bazely 2003).

An important point to mention is that not all introduced plant species that become established, are regarded as having only negative impacts on plant communities (Myers & Bazely 2003). Most introduced species have not become invasive and, in fact, from a biodiversity perspective, introductions have enriched some floras such as that of Central Europe (di Castri 1989). In addition, introduced species may have beneficial properties as potential medicinal plants (e.g. Ghisalberti 2000) or in agricultur (e.g. Roder *et al.* 1997, Norgrove *et al.* 2000). An example of such a plant is *Lantana camara* L., valued as an ornamental (Cronk & Fuller 1995). Its ability to dominate new environments was not recognized until after it had become widely spread around the world (Myers & Bazely 2003), see Figure 1.3. *L. camara* is on various lists of the top invasive woody weeds in the world (Cronk & Fuller 1995).

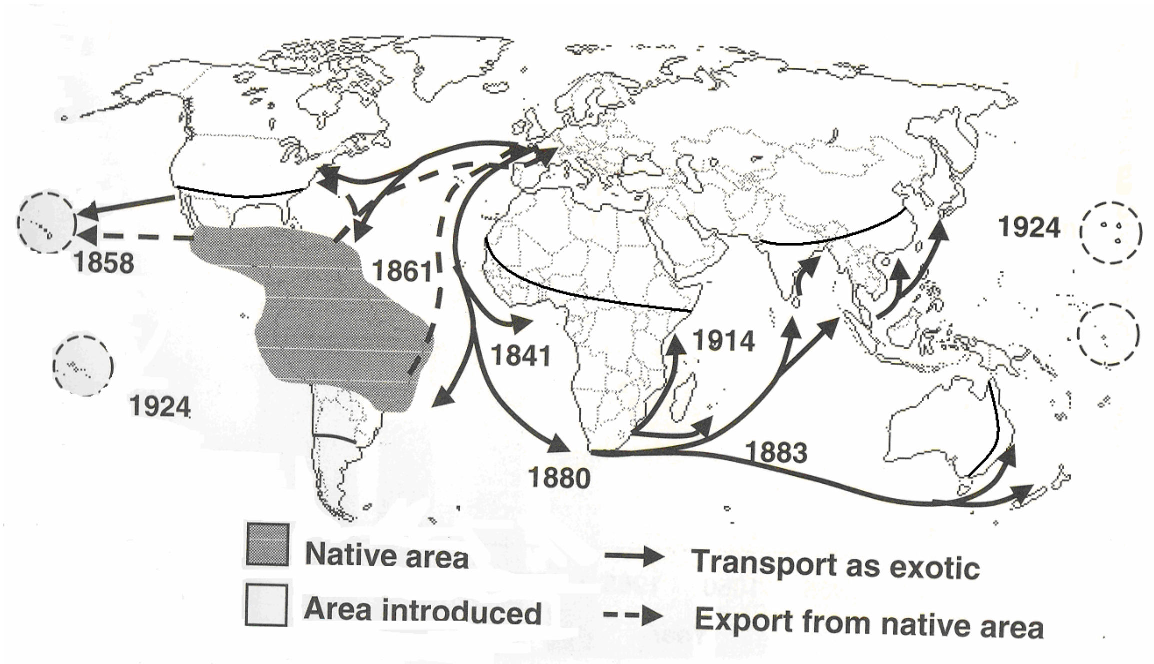


Figure 1.3 Map showing years of transport and introductions of the shrub *Lantana camara*, around the world, from its native continent of South America. Invaded areas are delimitated by solid lines. From Myers & Bazely (2003), originally modified from Cronk & Fuller (1995).

Lantana camara contains iridoid glycosides that may have a potential for use as anti-cancer drugs (Ghisalberti 2000). In addition to its medicinal properties, *L. camara* is widely valued as a popular garden plant with several different brightly coloured cultivars. Moreover, agricultural benefits of *L. camara* have been reported, among them improved soil fertility and slowed soil erosion (Ghisalberti 2000). Potential negative impacts of *L. camara* might be its potential to outcompete native/endemic species.

Several theories about plant invasions have been made. Already, Charles Darwin (1859) suggested that plant species can invade native communities because they are avoided by herbivores in their new habitats. The natural enemy hypothesis states that introduced plants do well because they lack specialist natural enemies. The biotic resistance hypothesis states that introduced species are superior competitors because native herbivores apply little biotic resistance to the invasion of plants (Myers & Bazely 2003).

Observations of plants being larger in exotic than in native habitats led Blossey & Nötzold (1995) to hypothesize that plants expended less energy on antiherbivore defences when they lacked specialist herbivores and therefore could devote more resources to growth. Evidence, reviewed in Myers & Bazely (2003) does, however, not support this hypothesis.

Rejmànek (1996) found a decrease of alien species in the tropics compared to higher latitudinal ranges and therefore presented the idea of a higher resistance against invasion in the diverse tropical communities. In areas with a diverse community of native species, limiting resources are used more completely and these areas were supposed to be more resistant to invasion than low diversity areas (MacArthur & Wilson 1967, Pimm 1991). The field evidence of Stadler *et al*. (2000), however, points to a contradictory hypothesis. They found that zones in north-western Kenya with high richness of native species also had high richness of introduced species. Apparently species richness of native plant communities therefore does not increase (at least not always) the resistance to invasions, and alien plant species can even invade diversity hotspots.

Simberloff (1981) argued that introduced species generally do not affect the communities they invade. He concluded that introductions apparently tend to add species to a community rather than to cause extinctions. Simberloff was criticized by Herbold & Moyle (1986) because he ignored substantial density changes caused by introduced species. Pimm (1991) stated that communities sometimes are resistant to introduced species, but most often are not.

Williamson & Fitter (1996) defined four stages of plant invasion: (1) introduction, (2) escape and establishment, (3) naturalization and spread, and (4) achieving pest or weed status. The likelihood of a plant species passing through these stages will depend on both the life-history characteristics of the plant and the characteristics of the environment. They also introduced the statistical “Tens rule” for plant invasions: 1 in 10 of imported plants appears in the wild (introduced or casual), 1 in 10 of these becomes established, and 1 in 10 of the established becomes a pest.

The introduction and invasion of exotic species might be studied as a special case of the “Equilibrium Theory of Island Biogeography” developed by MacArthur &

Wilson (1967). The introduction of non-native species raises the immigration rate and the effects of exotic species may increase the extinction rate, if they harm native species. Increased immigration of invasive species can therefore reduce the number of species if it causes extinction, or it can increase the number of species if the extinction rate remains unchanged.

It is widely recognized that most non-indigenous plants are not invasive (Lockhart *et al.* 1999). The problem is that usually the invasiveness of those species that do become aggressive weeds could not be predicted (Mullahey *et al.* 1998). Three clear lessons from past introductions might be learned:

1. The worst weeds were deliberately introduced and then escaped. Nearly all introductions of woody plants that have become invasive were introduced by horticulturalists, botanists, foresters, agroforesters or gardeners. One paradox that needs to be explicitly addressed is that many of the attributes of invasive species are precisely those characteristics favoured by the horticulture industry (White & Schwarz 1998).

2. Many invasive species have been repeatedly introduced without monitoring of the ecological consequences of the initial introductions (Myers & Bazely 2003).

3. Alterations of disturbance regimes are frequently associated with the establishment and spread of introduced species (Lozon & MacIsaac 1997).

1.3 Why do some species become invasive?

The rapid spread of species is generally achieved by connections between habitat patches. These are considered to promote the movement of organisms, and thus preserve biodiversity. These corridors may also facilitate the spread of invasive species, particularly if they are associated with long edges of disturbed habitat (Myers & Bazely 2003). Roads are particularly good corridors for the movement of non-native species; they alter conditions, stress native species, and allow easier access of humans and other vectors of plant dispersal (Trombulak & Frissell 2000). Disturbance associated with road construction provides a fertile environment for many weed species and the movement of seed on construction equipment is an excellent dispersal mechanism. In other words, disturbance can allow colonization of a plant community by different plant species (Canham & Louchs 1984).

How disturbance actually facilitates plant establishment is less well known. The creation of open patches for seedling establishment may be the most important factor. Disturbance such as fire (see below, under 1.3.1) and ploughing might also increase the available mineral resources and facilitate sun exposure. Selective grazing could reduce the competitiveness of palatable, native plants and facilitate the establishment of less palatable exotic species (Myers & Bazely 2003). That introduced species are able to invade and establish a new plant community indicates (Myers & Bazely 2003): (1) that there are empty niches in the native community, (2) that the introduced species itself creates a new niche or, (3) that the introduced species is a superior competitor able to respond to disturbance or utilize resources better than existing species.

Factors such as nutrients, seed number, and disturbance can influence the invasibility of communities and the success of invaders. In addition, the species diversity of a community could have an influence if invasion promoters such as pollinators, soil fungi, nitrogen fixing bacteria, or organisms that disperse seeds are included (Myers & Bazely 2003).

Life history traits such as seed size, seed dormancy, survival of seeds, their successful germination, growth pattern and the size or age at first reproduction will influence whether an introduced species might become established in a new area, and whether it will outcompete native plants. The traits regulate the plant species population dynamics over time (Figure 1.4). The dispersal of seeds determines the plants distribution (Myers & Bazely 2003).

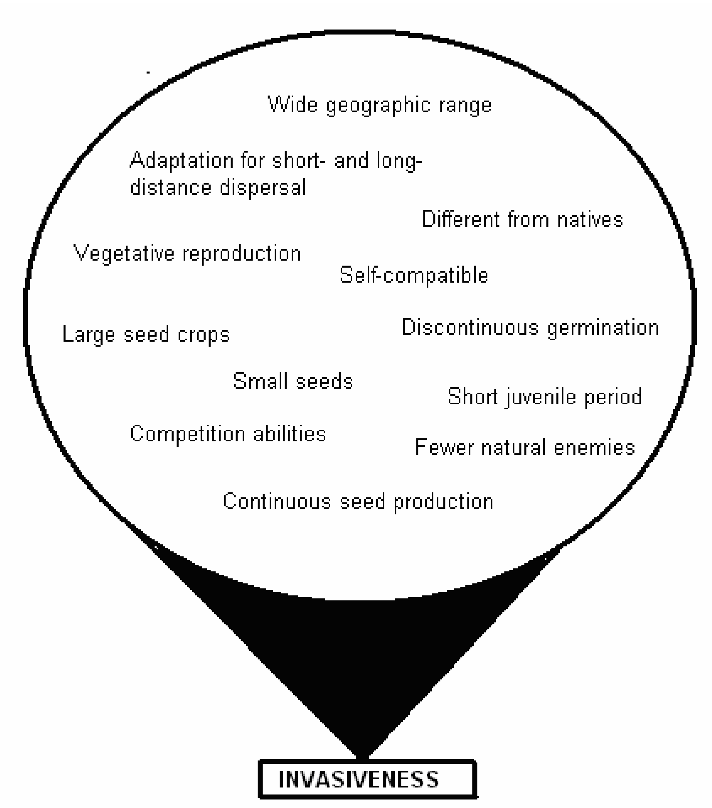


Figure 1.4 Invasive plant species often show some of these characters. Modified from Rejmànek (1996) and Baker (1974).

The period before first reproduction and the growth rate determine how quickly a plant population can grow. Characteristics of plant survival will determine the persistence of a species in a habitat, while the growth form and root structure of a species will influence its ability to compete. Characteristics that influence whether plants outbreed, inbreed or reproduce asexually will determine the level of genetic variation that they maintain, and thus how rapidly they might respond to changing selection regimes and varying environmental conditions. Self compatible species may be more efficient to establish populations after dispersal. The persistence of seeds in the soil (seedbanks, see below under 1.3.2) can buffer plants against temporarily poor conditions (Myers & Bazely 2003).

Baker (1974) proposed that the following predisposing characteristics might act either alone or together to increase the probability of a plant being invasive. These include 1) rapid initial growth rate and expansion of the root system, 2) ability to interfere with the growth of neighbouring plants, 3) high seed output under optimum conditions, with some seed produced under unfavourable conditions, 4) morphological and/or physiological similarity to native species, and 5) breeding system which allow self-pollination and outcrossing.

1.3.1 The impact of fire on invaders

Whether fire facilitates invasions by introduced species is disputed (Myers & Bazely 2003). Fire causes extinction of species, but recovery from fire is a balance between immigration and extinction (Collins *et al.* 1995). In general fire has an important impact in shaping biodiversity patterns in Africa (Getachew Tesfaye *et al.* 2004).

Vegetation in East African savanna areas often burns at least once a year, usually following the onset of the dry season. Subsequent fires are generally patchy because of the discontinuity of fuel, differences in relative moisture and wind intensity (Menassie Gashaw & Michelsen 2002). Consequently plants and their seeds are exposed to different temperature ranges (Herranz *et al.* 1998). After a long time of evolution, most of the natural vegetation in east Africa has evolved seeds adapted to tolerate annual fires. The fire regimes decide whether fire is beneficial or detrimental to seed germination and plant regeneration (Skoglund 1992).

The fires might break down dormancy caused by impermeability of seed coats (Whelan 1986). This can be done artificially with dry heat treatment which is analogous to heating by vegetation fire (Martin *et al.* 1975). This has been done in many scientific experiments, and is particularly good for hard-coated, water-impermeable seeds (Gonzalez-Rabanal & Casal 1995). Too high temperature over a too long period will be lethal for all seeds. Knowledge of germination responses of seeds to high temperatures, to charred wood and to smoke derivatives may indicate how to employ fire as a management tool in maintaining plant diversity in fire-prone ecosystems (Keith 1997).

1.3.2 The importance of seed banks for invaders

Many invasive plants are efficient seed producers. This contributes to their local reproduction, as well as their potential for dispersal (Gross 1990). A persistent seed bank might be one of the characters which distinguish an invasive species, because seed dormancy provides a strategy for plants to cope with temporal environmental variation. It is the accumulation of seeds in the soil that forms the seed bank (Myers & Bazely 2003). A viable seed bank is important since no plant community is entirely undisturbed and regeneration by seeds is important to maintain the plant cover (Thompson 1993, Larsson 2002). The existence of a seed bank spreads germination over good and bad years and is an excellent adaptation for plants living in unpredictable environments. Seed banks have strong impacts on the persistence of invasive plants (Myers & Bazely 2003). Soil disturbance can stimulate germination of seeds in the seed bank.

Seed banks optimise long-term population growth rate and delay possible extinction time (Kalisz & McPeek 1992). Seed banks are shown to be larger in disturbed soil than in undisturbed soil (Hansen 2003). The presence or absence of a seed bank is most likely an important factor controlling natural re-vegetation after disturbances, and seed banks can prevent the invasion of new species, by shortening the period for successful establishment of aliens on uncolonized soil (McGraw & Fetcher 1992).

1.4 Control of introduced plants

Applied biologists and landscape managers are challenged by the need to conserve the diversity and integrity of natural plant communities in the face of invasive introduced species, increased disturbance and changing environmental conditions. While eradication of introduced species may be considered, the reality is that once a species has reached the point where it is recognized as a problem, eradication might not be achievable (Myers & Bazely 2003).

Classical biological control is undertaken by the introduction of natural enemies in an attempt to eradicate exotic pests. If introduced weeds have a competitive advantage because they lack natural enemies, then establishing the natural enemy complex might be a way of levelling the playing field (Myers & Bazely 2003). Successful control-programs represent only a fraction of those that have been attempted, but biological control is the only potential solution for many invasive weeds (Myers & Bazely 2003). Only with considerable investments of time and money, is control likely to be successful (Goodall & Erasmus 1996, Mack *et al.* 2000).

Biological control has both costs and benefits, but the need for some kind of control increases, as more plants are introduced to new areas. The practice of biological control increases the number of introduced species, and in this way has the potential to increase the ratio of exotic to native species. On the other hand, a high density of an established invasive weed can, as mentioned earlier, suppress native vegetation and reduce local biodiversity.

The first, and one of the most successful biological control programs, was the control of the prickly pear cactus (*Opuntia stricta*) in Australia by the Cactoblastis moth (*Cactoblastis cactorum*) (Straw & Sheppard 1995). Temperate species of annual plants have rarely been successfully controlled (Myers & Bazely 2003, see Figure 1.5). Over 20 species of natural enemies have been introduced in largely unsuccessful attempts to control *Lantana camara* (Broughton 2000).

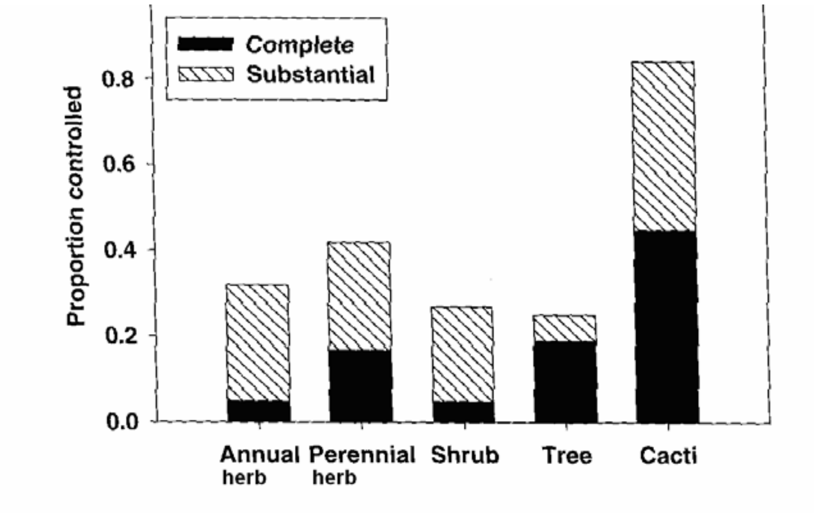


Figure 1.5 Proportion of biological control projects that achieved substantial or complete control of the alien species. Data from Straw & Sheppard (1995).

Targeted control methods of physical removal include cutting and pulling a problem invader. Pulling plants can result in soil disturbance and trampling which can facilitate recovery of plants from a seed bank (Myers & Bazely 2003). Cutting is clearly much more effective (Ussery & Krannitz 1998), unless underground runners or suckers are present.

Non-targeted control methods include burning and chemical control. Burning has been recommended as a management tool for suppressing non-indigenous species. Prescribed burning schedules can vary as to season, fuel load, and frequency. It is vital to carry out experiments and monitoring to evaluate the outcome of burning as a control tool (Myers & Bazely 2003).

A number of issues are associated with the use of chemical control. First is the possibility that non-target organisms will be affected (Rea & Storrs 1999). Additional problems are the need to have an approved herbicide (e.g. Goodall & Erasmus 1996), and the need to avoid the evolution of resistance. The economical situation should also be addressed before this control method is used.

1.5 Benshangul Gumuz National Regional State

Benshangul Gumuz National Regional State (BGNRS) situated in the Western Ethiopian escarpment was chosen as the study site, because the thesis is part of a larger project (Tesfaye Awas Ph.D study: “Plant biodiversity and ethnobotany in the lowlands of Western Ethiopia”). One of the reasons why this area is being investigated is that it exhibits a high degree of plant endemicity. In addition, the area is so far not very well studied. A few pilot studies have revealed several species new to science (Sebsebe Demissew *et al.* 2005). Much of the natural vegetation is still nearly intact, but the area has generally become more accessible because of increased road development. Sebsebe Demissew *et al.* (2005) stated that an increasing knowledge of the unique biodiversity in this area might contribute to conservation of the vegetation and flora. The spread of any introduced plant species in this kind of environment might have serious consequences.

The flora of BGNRS consists of at least 950 species, of which at least 27 are endemic or near-endemic (Sebsebe Demissew *et al.* 2005). More accessible regions with passable roads and other infrastructure have been better investigated than those without these facilities. The development (including road construction, immigration etc.) in the area may present a number of threats to the biodiversity. These changes could be (Sebsebe Demissew *et al.* 2005):

(1) Loss of wetlands by draining, which would mean loss of habitat for the many rare orchids and other wetland species, including endemics.

(2) Unsustainable development of the woodlands and bamboo-thickets by uncritical fuelwood cutting, charcoal-burning and mining, which may harm future sustainable exploitation of the woody vegetation by the local people and might threaten some of the woody endemics.

(3) Unsustainable development of agriculture and mining in the wooded grasslands, which might threaten some of the rare grassland and woodland species.

(4) Change from traditional slash and burn agriculture to large scale cultivation.

A 5th point is here added to the list:

(5) Introduction of alien invasive species.

1.6 Introduced ornamentals in Benshangul Gumuz National Regional State

Most gardens in Benshangul Gumuz in Western Ethiopia are dominated by useful plant species, e.g. vegetables, spices, tobacco, or species which give shade. Some gardens also includes several ornamentals (as in Figure 1.6). *Tagetes patula*, *Canna indica* L., *Lantana camara* L., *Zinnia elegans* Jacq., *Datura metel* L., and *Brugmansia arborea* (L.) Lagerheim are the most common species, and they are all introduced. *Tagetes patula* and *Zinnia elegans*, both in the family Asteraceae, are the only introduced ornamentals which have been noticed outside gardens, spreading into woodlands (Tesfaye Awas, pers. comm.).

Figure 1.6 *Tagetes patula* and *Zinnia elegans*, introduced ornamentals in a garden in the village Finote Selam, Western Ethiopia. Photo A.B.S. 12 Oct. 2005

Tagetes patula is originally native to North and South America, but is introduced as an ornamental all over the world. *Tagetes patula* was first observed naturalized in Western Ethiopia in 2001 (Tesfaye Awas, pers. comm.). It had then spread into more or less natural vegetation and grazing lands around Mandura Village. In October 2004, it was observed (again by Tesfaye Awas) to have increased its distribution to several sites where it was not registered in 2001. It was concluded that the impact on natural vegetation should be further studied to find out whether it might become a harmful invader in farmlands and natural vegetation in the Benshangul Gumuz region.

Zinnia elegans is introduced to Africa as an ornamental from North America. It is not known to be invasive, but it has been recorded as escaped in the Shewa Region (Mesfin Tadesse 2004). The two mentioned Asteraceae species share a lot of characteristics, and it was concluded that it would be interesting to compare them with an Asteraceae species with different characters in further studies. *Bidens prestinaria,* a common indigenous Asteraceae species was chosen as such a species.

Studies on characteristics such as seed production, seed dispersal, soil seed banks, germination behaviour of seeds and the ability of broken or eaten plants to resprout, can provide insight into the regeneration capacity of a given species and thereby its invasive ability (Hailu Shiferaw *et al.* 2004). Invasive species are, however, often appreciated by the local people, and it is therefore necessary to have a good dialogue and discuss positive and negative effects for each given species with representatives from the local people.

1.7 Aims of the study

General objective

To study ecological and biological factors that may facilitate invasion by *Tagetes patula* L. (Asteraceae) in Western Ethiopia. The introduced ornamental *Zinnia elegans* and the native species *Bidens prestinaria* will be included in some of the tests for comparison.

Specific objectives

The following questions will be addressed in this thesis.

1. Does the introduced ornamental species *Tagetes patula* and *Zinnia elegans* escape from gardens?

2. Which habitat types are vulnerable to invasion of *Tagetes patula*?

3. Do the ornamental species of Asteraceae have life history traits that give potential for invasiveness?

4. Could fire facilitate or control the invasion of natural vegetation by *Tagetes patula* and *Zinnia elegans*?

2. Materials and methods

2.1 Study site

The fieldwork was conducted in Benshangul Gumuz National Regional State (BGNRS) in Ethiopia (Figure 2.1), a country situated in the Horn of Africa. The country consists of a massive highland complex of mountains and plateaus divided into the eastern (Harrar) and western (Central Ethiopia) plateaus, by the Great Rift Valley. Lowlands are situated along the periphery. The western plateau is more extensive and rugged than the eastern plateau, and it is divided into a northern and a southern section by the Blue Nile (Abay) valley (http://countrystudies.us/ethiopia/41.htm, Solomon Tadesse *et al*. 2003).

Figure 2.1 The Federal Democratic Republic of Ethiopia with updated delimitation of recently defined "National Regional States".

Volcano-sedimentary belts accreted together during the East- and West-Gondwana collision (Neoproterozoic, 900-500 Ma), this contributed to a Precambrian crystalline basement in Ethiopia. The basement contains several minerals, both precious and rare metallic and industrial minerals such as gold, platinum, nickel, copper, iron, chromium, haolin, feldspar, clay, asbestos, and talc, and the more common rocks marble, limestone and granite (Solomon Tadesse *et al.* 2003).

Altitude-induced climatic conditions form the basis for three environmental zones; the *dega* (cool), the *weina dega* (temperate), and the *kola* (hot). The terrain in the cool zone is generally above 2,400 meters in elevation. Lower areas, between 1,500 and 2,400 meters in elevation, constitute the temperate zone. Daily highs here range from 16-30 ºC. The hot zone consists of areas where the elevation is lower than 1,500 meters (http://countrystudies.us/ethiopia/41.htm). The study area of this thesis is situated in the temperate zone.

The fieldwork was undertaken from September to October 2005. BGNRS lies in Western Ethiopia (Figure 2.1 & 2.2), and is bordered by the Sudan in the west, the Oromiya Region to the South/East and the Amhara Region to the North/East. At the time the field work was carried out, the rain period had lasted for about 3 months, and the vegetation was accordingly luxuriant.

The topography (Figure 2.2) is characterised by a rolling terrain, sloping relatively gently but sometimes dropping steeply from an average of ca. 1800 m in the Ethiopian highlands (to the east), to 500-700 m close to the lowlands of Sudan (Sebsebe Demissew *et al.* 2005).

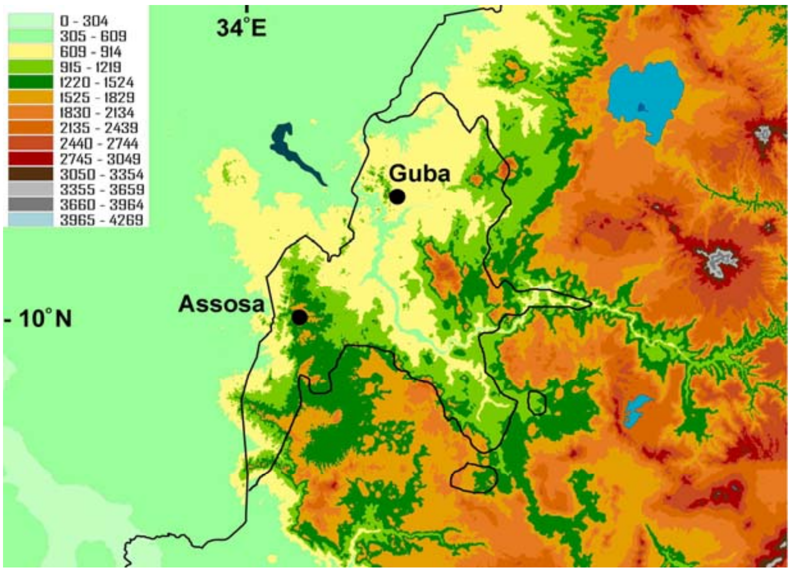


Figure 2.2 Topographical map with BGNRS delineated in black.

Average rainfall (1200 mm in Bulen, for locality see Figure 2.10), is reduced towards the North and the West to about 800 mm. The mean annual temperature varies from 20º C to 35º C. The variation is strongly correlated with altitude (Sebsebe Demissew *et al.* 2005). In the study area the rainy season is about three months long, from mid-June to mid-September followed by a dry period. More detailed climatic data are presented for one village in the study area, Bulen (Table 2.1).

Table 2.1 Summary of weather conditions during the year in Bulen (10º 41`N, 36º 06`E, Alt. 1450). All data from National Meteorological Agency of Ethiopia. Temperatures in º C and precipitation in mm.

Bulen;	**Aug.**	**Sept.**	**Oct.**	**Nov.**	**Des.**	**Jan.**	**Feb.**	**Mar.**	**Apr.**	**May.**	**Jun.**	**Jul.**
Temp.												
Monthly Mean 1980-2004	19,3	19,7	20,1	19,8	19,3	19,7	20,1	19,9	19,9	20,3	21,2	19,6
Min. 1980-2004	14,4	14,2	14,1	12,2	11,2	11,0	12,2	14,4	16,2	14,7	14,7	14,7
Max. 1980-2004	24,2	25,1	26,0	27,5	28,6	29,5	30,1	31,6	31,2	29,4	26,0	24,5
Precip.												
Monthly Mean 1980-2004	369	261	148	18	1	1	1	12	31	153	254	344

The geology of the area comprises outcrops of old Precambrian rocks. Deep clayish red soils predominate in most of the zone south of the Blue Nile (Abay) river. These have good physical properties with general agricultural potential (Sebsebe Demissew *et al.* 2005). The predominant soils north of the Abay river are chemically poorer and have a more limited agricultural potential. The western geological formation in BGNRS holds rich mineral deposits of gold, copper, lead, and zinc. In addition there are important occurrences of marble, which to some extent is utilised.

BGNRS has a population density of 10.9 individuals pr km^2, while the overall number for Ethiopia is 57.7. Even though the area is less populated than others, the population of the BGNRS is expected to double within 2030 (Bureau of Planning and Economic Development 1998), and elsewhere a similar or slightly lower increase seems likely.

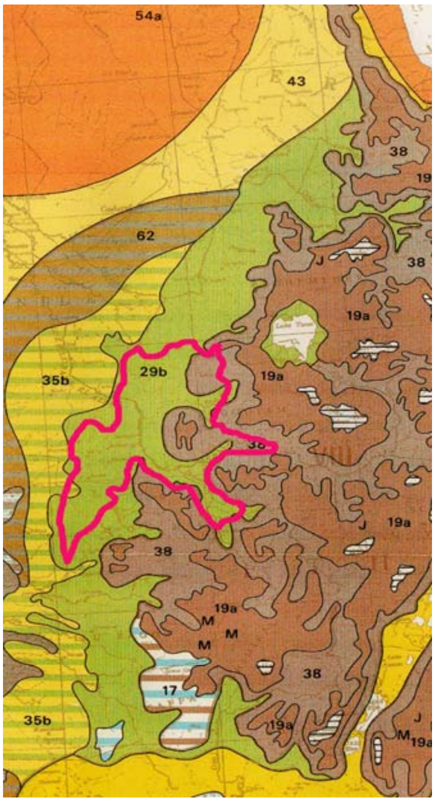


Figure 2.3 Section of White's (1983) vegetation map with BGNRS outlined in red.

The destruction of forest and woodland resources has been less in BGNRS than in other parts of Ethiopia. This is due to the, so far, low population density and the general inaccessibility of the region (Sebsebe Demissew *et al*. 2005). About 60 % of the region is covered with different types of forests, woodlands and bamboo thickets. The common woodland type is dominated by small to moderately sized trees with fairly large deciduous leaves, like *Terminalia* spp., *Combretum* spp., and *Lannea* spp., as well as *Entada abyssinica* Steud. Ex A. Rich., *Erythrina abyssinica* DC., *Strychnos innocua* Del., *Anogeissus leiocarpus* (DC.) Guill. & Perr., and *Stereospermum kunthianum* Cham. Particularly interesting is the common occurrence of the solid-stemmed lowland bamboo, *Oxytenanthera abyssinica* (A. Rich.) Munro. The ground cover is mainly a tall stratum of perennial grasses, including species of *Cymbopogon*, *Hyparrhenia*, *Echinochloa*, *Sorghum* and *Pennisetum* (Sebsebe Demissew *et al.* 2005). White (1983) defined this vegetation type as "Undifferentiated woodlands (Ethiopian type)" (Figure 2.3). Vegetation type 29b - "Undifferentiated woodlands (Ethiopian type)" dominates the area. In the eastern part vegetation type 38 –"East African evergreen and semi-evergreen bushland and thicket" and vegetation type 19a –"Undifferentiated montane vegetation (Afromontane region)" are also found (White 1983).

The woodland is part of a zone with a high fire frequency. The vegetation has been burning annually for such a long time that the plants show several adaptations to fire, and the vegetation must be assumed not to be adversely affected by controlled annual fires (Jensen & Friis 2001, Menassie Gashaw & Michelsen 2002).

2.2 Target species

2.2.1 *Tagetes patula* L.

The genus *Tagetes* (popular genus name: Marigold) includes about 50 species indigenous from the south-western United States to Argentina. The greatest diversity of the genus is found in south-central Mexico. Three species of *Tagetes* are introduced to Ethiopia. Two of them, *Tagetes patula* L. ("French marigold") and *Tagetes erecta* L. ("American or African marigold"), are cultivated ornamentals. The last one, *Tagetes minuta* L. is a noxious weed of farmland, waste places and roadsides, and is found all over Ethiopia (Mesfin Tadesse 2004).

All species of *Tagetes* are annual, aromatic herbs with mainly opposite leaves. Capitulas are radiate (containing an outer ring of ray florets surrounding the disc florets), and have fused phyllaries. Each phyllary contains oil glands that give the plants their characteristic odour. The ray florets are fertile and showy. The disc florets are fertile, but not so showy. Cypselas are brownish to black with a pappus of 3-10 unequal scales (Mesfin Tadesse 2004). Yellow, orange, golden or bicoloured flowers are exposed either well above the fine-textured, dark green foliage or tucked in with the foliage, depending on the cultivar (Gilman 1999).

Tagetes species contain carotenoids and lutein, giving the flower the characteristic yellow to red colours. The chemical compounds can be used for dying. Marigolds are recorded to have been used by the Aztec people to treat hiccups. It has been proposed that in the 16th century, native marigold seeds were taken from the Aztecs by early Spanish explorers to Spain where the plants were cultivated in monastery gardens. From Spain, marigold seeds were transported to France and northern Africa. A particularly large-flowered species, *Tagetes erecta*, became naturalized in North Africa. Seeds were collected and once reintroduced to Spain where its flowers were named Flos Africanus due to its mistaken origin. Several hundred years after their initial introduction from America to Europe and Africa, marigolds were introduced to American gardens. Sweet peas and asters had been popular flowers in the US, but both of them were becoming beleaguered by disease and declining overall performance. Marigold seeds were first featured in commercial catalogues in 1915. Since the 1920s many new marigold varieties have been bred. The principal producers of *Tagetes* as ornamentals today are Mexico, Peru, Ecuador, the United States, Spain and India (http://www.wildflavors. com/index.cfm).

Tagetes patula differs from *Tagetes minuta* and *Tagetes erecta* in size. *T. minuta* is smaller, and has only 2 to 3 unshowy ray florets. *T. erecta* is larger, and has more and larger ray florets. The ray florets are showy and often yellow. It is characterized as a garden ornamental, rarely found as an escape in fields in a few regions of Ethiopia. *T. patula* is more common than *T. erecta* (Mesfin Tadesse, 2004). Other important diagnostic characters of *T. patula* are given in Table 2.2.

Tagetes patula (Figure 2.4) is a garden ornamental, also found as an escape in fields and on river banks between 1600 and 2440 m in Ethiopia. The species is native of Mexico and Central America, but widely cultivated and naturalized in many temperate countries (Mesfin Tadesse 2004).

Tagetes patula is used in some parts of Africa and India to colour foods (butter, cheese, etc) and as a fabric dye. In addition the leaves are used as a seasoning in central Africa, and the dried flowers are sometimes used to adulterate saffron (http://www.foodreference.com/html/ffrenchmarigold.html). The plant grows in full sun and is according to Gilman (1999) not known to be invasive.

a) *Tagetes patula* **b) *Zinnia elegans*** **c) *Bidens prestinaria***

Figure 2.4 Illustrations of the three study species; a) *Tagetes patula*, b) *Zinnia elegans* (Photos: A.B.S.) and c) *Bidens prestinaria* (Photo: Tesfaye Awas).

2.2.2 *Zinnia elegans* Jacq.

The genus Zinnia includes about 23 species. They are mainly native to North America. Only two species, *Zinnia elegans* Jacq. and *Zinnia peruviana* (L.) L. are known to occur in Ethiopia (Mesfin Tadesse, 2004).

The plants in the genus are annual or perennial herbs, sometimes shrubby, with a slender or thickened taproot. The leaves are always opposite, simple, entire and sheathing the stem. The capitula is solitary and radiate. The phyllaries are placed in three or more series, the outer progressively shorter. Ray florets are female, fertile, 1-3-lobed and may be white, yellow, orange, red, purple or lilac in colour. Disc florets are bisexual and also fertile. Ray- and disc cypselas have different shape, and the pappus consists of 1-3 awns, sometimes absent in ray cypselas.

Zinnia elegans (Figure 2.4) is taller than *Zinnia peruviana*, with an up to twice as large capitula. *Z. peruviana* has reddish or brownish-red ray florets, while the ray florets in *Z. elegans* have other colours (see Figure 2.5). *Z. elegans* has disc cypselas without a pappus, while *Z. peruviana* has disc cypselas with a single long pappus scale. *Z. peruviana* is, in contrast to *Z. elegans,* a widespread, weedy species (Mesfin Tadesse 2004). More morphological details of *Zinnia elegans* are found in Table 2.2.

Figure 2.5 Ray florets of *Zinnia elegans*, in different colours. The flowers were grown in the Phytotron at the University of Oslo (UoO), Blindern. Photos: A.B.S. 15 Mar. 2006.

Zinnia elegans is a widely cultivated ornamental plant throughout the world. It has according to Mesfin Tadesse (2004) not yet become naturalized in Ethiopia, and occurs only in gardens or rarely as an escape from gardens along roadside ditches in the Shewa region in altitudes from 1500 to 2400 m.

2.2.3 *Bidens prestinaria* (Sch. Bip.) Cufod.

The genus *Bidens*, with a world-wide distribution, contains about 340 species. The tropical and subtropical regions of North and South America and Africa are diversity centres. About 20 species are found in Ethiopia (Mesfin Tadesse 2004).

The genus consists of erect, annual or perennial herbs, or shrubs. The leaves are opposite or rarely whorled with different shapes. Outer phyllaries are green while inner phyllaries are grey- or orange-striated with scarious margins. The outer ray florets are yellow or white, rarely with orange blotches. The disc florets are yellow, orange or brownish-orange and bisexual. Cypselas are dorsiventrally compressed, or 3-4 angled, sometimes narrowed and elongated above.

Bidens prestinaria (Figure 2.4) differs from the 11 perennial *Bidens* species found in Ethiopia by being annual. *B. prestinaria* has a 2.5-4 cm wide capitula at anthesis, drooping in fruit. The leaf blade is ovate to tri-pinnatisect, and the cypselas have flat wings. Only four of the nine annual *Bidens* spp. of Ethiopia have cypselas with flat wings. *B. borianiana* (Sch. Bip. ex Schw.) Cufod. has a similar cypsela, but can be separated from *B. prestinaria* by the large size of the capitula, which stay erect in fruit. *B. pilosa* L. has white ray florets (if present), and *B. biternata* (Lour.) Merr. & Sherff. ex Sherff. has a smaller capitula than *B. prestinaria* (Mesfin Tadesse 2004). More morphological details of *Bidens prestinaria* can be found in Table 2.2.

Bidens prestinaria grows in short grassland, gentle mountain slopes, and on river and stream banks. The species is also extending into the margins of arable land and is sometimes found along roadsides from 950 to 2850 m in Ethiopia (Mesfin Tadesse 2004). *Bidens prestinaria* is used in honey production by the local people. The areas where the species grows are burnt annually, and the plant is heavily grazed by domestic animals.

Table 2.2. Comparison of the three study species: *Tagetes patula*, *Zinnia elegans* and *Bidens prestinaria.*Illustrations of the three species is found in Figure 2.4.

Plant species;	*Tagetes patula*	*Zinnia elegans*	*Bidens prestinaria*
Introduced ornamental:	Yes	Yes	No
Native habitat:	Mexico, Central America	North America	Ethiopia, Sudan
Annual:	Yes	Yes	Yes
Stem:	Rooting from the lower nodes, dark green becoming brownish	Not rooting from the lower nodes, green becoming yellowish to purple	Rooting from the lower nodes, green becoming brown
Leaves:	Alternate, imparipinnate, 1-10 cm long, margins serrate	Ovate to lanceolate, sessile or subsessile, up to 8x4 cm, margins entire	Simple, ovate to tri-pinnatisect blade up to 20 cm
Capitula:	Solitary at the end of branches, not nodding in fruit	Solitary, at anthesis, not nodding in fruit	Solitary at the end of branches in lax paniculate cymes, nodding in fruit,
Phyllaries:	Fused, 5-7 lobed at apex, with glands	Multiseriate, green or dark-banded, no glands	Outer: green, 3-veined Inner: grey to orange with yellow scarious margins, no glands
Ray florets:	6-8, yellow on the upper surface, dark-orange/red on the lower, often reddish-brown throat	8-20, yellow, orange, pink, red, scarlet, lilac, purple or white on both sides	8, Yellow on both sides with orange blotches at middle and at base
Disc florets:	Numerous, yellow, corolla 5-lobed	Numerous, yellow, black on outside, corolla 5-lobed	Numerous, yellowish-brown, corolla 5-lobed
Cypselas:	Black or brownish, hispidulous, 7-9 mm long	Brown, 4.5-6mm long, dimorphic, ray cypselas oblanceolate to cuneate, disc cypselas laterally compressed	Dark brown, 3-9x0.8-3.5 mm incl. wings, irregular in size, longest in the centre excl. wings, flat incl. wings in the periphery
Pappus:	5 basally fused scales	Absent	Absent, modified to aristae

2.3 Methods

2.3.1 Mapping distribution of escaped ornamentals in Benshangul Gumuz

A relatively large area of western Ethiopia was searched for populations of *Tagetes patula* and *Zinnia elegans* to map the distribution of escaped plants. The search was done from a car. Due to the showy colours of the plant, it was easily observed at least 100 m from the roadside. When plants were spotted, GPS, altitude and habitat type were recorded. Plants observed in gardens were also registered (for *T. patula* only).

The registration of plant populations, occurring along the roadsides, was done from Nekemte to Assosa, from Nekemte to Guba/Mankush and from Guba/Mankush to Addis Ababa (for detailed coordinates see Table 2.3).

Table 2.3 GPS coordinates and altitudes for some registration points in Western Ethiopia. See also Figure 2.2

Location:	GPS:	Altitude:
Nekemte	09 º 16.38\` N, 36 º 31.05\` E	1547m
Assosa	09 º 51.49\` N, 34 º 41.30\` E	1465m
Guba/Mankush	11º 16.15\` N, 35º 17.89\` E	892m
Addis Ababa	09° 21.4\` N, 38° 46.8\` E	2400m

ArcView version 9.1 was used to make a map with the spotted escaped and garden plant populations plotted in two different colours. ArcView is a full-featured GIS software for visualizing, analyzing, creating, and managing data with a geographic component (http://www.esri.com/software/arcgis/arcview/index.html).

2.3.2 Seed production

To determine the number of seeds produced in one capitula during the flowering period, inflorescences were collected from random mature plants. A total of 100 *T. patula* capitulas were collected, from the area outside Bulen (Table 2.4). After collecting, the capitulas were opened, and the number of cypselas per head was counted. A total of 20 *Bidens prestinaria* capitulas were collected, and the number of cypselas in each head was counted to find the overall mean number. The plant heads were collected outside Dibate (Table 2.4).

In addition, all *T. patula* and *B. prestinaria* plants in the association analysis quadrats were collected, and the number of capitulas on each plant was counted.

Table 2.4 GPS coordinates and altitudes for the locations where counted *T. patula* and *B. prestinaria* capitulas were collected. See Figure 2.10

Location:	**GPS:**	**Altitude:**
Bulen	10º 41`N and 36º 06`E	1450m
Dibate	10º 47`N and 36º 16`E	1575m

2.3.3 Information from local people

To investigate the role of *Tagetes patula* in native peoples life, people were asked questions about the positive and negative effects of the plant, when it arrived, if it was of any use, if it was eaten by animals, if it was actively or passively spread etc. (Figure 2.6). People present in the areas where *T. patula* had escaped were randomly selected to be interviewed. The information obtained was used to decide which interest people had in the plant, and if it had an acute effect on peoples everyday life. *Zinnia elegans* was rare as escape, and was not included in the study.

People in areas dominated by *Bidens prestinaria* were interviewed about that species` role in the community. This was done to compare different people's opinion of an introduced versus a naturally existing plant species.

Figure 2.6 Three local people (to the right) were interviewed about *Tagetes patula*. Photo: A.B.S. 29 Sept. 2005.

2.3.4 Fire simulation experiment

Diaspores from *Tagetes patula, Zinnia elegans* and *Bidens prestinaria* were collected from ripe specimens in different localities within the study area (see Table 2.5). All the diaspores were collected from naturalized or naturally existing plants. The areas were not regularly burnt, because of the localization close to grazed land and farm lands. All species could be found in disturbed roadside vegetation, and developed their seeds in the end of the rainy season, late October.

Table 2.5 Collection locality and collection date for the three study species included in the fire simulation experiment. See the areas on the map in Figure 2.10

Plant species:	**Collection locality:**	**Collection date:**
Tagetes patula	Bulen	08.10.2005
Zinnia elegans	31.2 km east of Assosa	04.10.2005
Bidens prestinaria	Dibate	07.10.2005

Diaspores were cleaned and sorted out by selecting the ones without evidence of insect and fungal damage (Menassie Gashaw and Michelsen 2002), and then the most mature diaspores were chosen for the experiment. Ten randomly chosen diaspores from each species were measured, to find the average length and weight of the diaspore. Before the selection, the diaspores had been stored for about two weeks in paper bags at room temperature.

The fire simulation experiment was carried out at the University of Oslo, in the Phytotron. About five weeks after collection, diaspores from each species were subjected to heat treatment according to six different prescriptions: 60, 90, and 120 ºC, for 1 and 5 minutes duration (treatments denoted: control, 60ºC/1 minute, 60ºC/5 minute, 90ºC/1 minute, 90ºC/5 minute, 120ºC/1 minute and 120ºC/5 minute, respectively). These are temperatures likely to be reached at the soil surface or the first few centimetres below ground in savanna fires (DeBano *et al.* 1998). Dry heat treatments were accomplished in an oven, with rapid insertion and removal of an aluminium tray containing the diaspores. For each species, a total of 350 diaspores in five replicates of sets of 10 seeds were used. Untreated diaspores were used as control (stored at 20 ºC), with the same number of replicates as that of the other, higher temperature treatments (Menassie Gashaw and Michelsen 2002). Treated and untreated diaspores were soaked in water on a filterpaper in a Petri dish. Ten diaspores were placed in each Petri dish, see Figure 2.7.

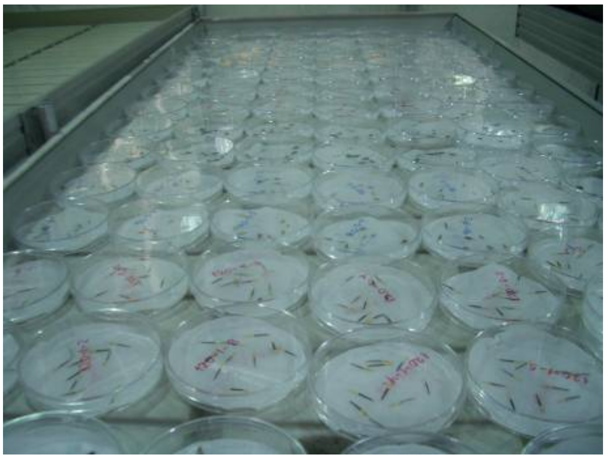


Figure 2.7 Petri dishes with water-soaked filterpaper. The diaspores were exposed to different heat temperatures. Photo: A.B.S. 28 Nov. 2005.

The diaspores were placed in a thermo regulated greenhouse with an average temperature of 26 ºC during the day (light, 12 h) and 20 ºC during the night. The filterpapers were moistened every day for the first month, and then every third day till the end of the experiment. Seeds were considered to have germinated when the radicle penetrated the seed coat. Germination was recorded daily the first two weeks, after this every third day. The germinated seeds were removed from the Petri dishes. The experiment ended after 18 weeks.

To evaluate the frequency of germination, the total germination in each treatment was presented in a scatter plot in EXEL. Germination periods for the three species were described separately in bar diagrams. The data were also incorporated in OneWay Anova statistics to find out if there were significant different germination fractions in the heat treatments. P-values were found in a Turkey pairwise comparison, in PAST (Hammer *et al.* 2006).

2.3.5 Germination experiments

Diaspores from *Tagetes patula*, *Zinnia elegans* and *Bidens prestinaria* were collected from the same localities as the diaspores used in the fire simulation experiment (Table 2.5). The diaspores were subjected to two different treatments; (i) diaspores were sown on top of the soil, only covered with a thin layer of sand (to deter flies and algae growth); (ii) diaspores were sown, covered with 1 cm soil and then covered with a thin layer of sand.

In each treatment, two replicate pots of 10 diaspores each were used. All diaspores were sown in 12 cm black pots with sterile humus soil/perlite mixes. All pots were watered, put under plastic sheets to keep moist during the first period, and placed on a table in a greenhouse with temperatures around 25ºC during the day (light) (12h) and 20ºC during the night (dark) (12h). The pots were watered every day, and the plastic was removed after the first germinations took place. Seeds were considered to have germinated when the plantlet could be seen above the soil (Figure 2.8). Germinating seeds were in the beginning of the experiment counted every day, and the height of the growing plants was measured every week.

Figure 2.8 *Tagetes patula* germination experiment in the Phytotron at UoO. Photo: A.B.S. 8 Nov. 2005.

After about six weeks each plant was replanted in a separate pot, to avoid competition. After three months the plants were moved into a closed phytotron room. The reason for this was that lights in the hallway outside the greenhouse most likely affected the plants so that they did not flower. The temperature was changed to ca. 25ºC during daytime (12 h), and 15ºC during the night (12h). The temperature was set to mean temperature during September, the flowering month in this part of Ethiopia. The plants were watered every day.

The data from the germination experiment was analysed in EXEL. Bar diagrams were made to illustrate differences in germination periods. Scatterplots were made to illustrate average height during the growing period.

2.3.6 Flower production in grazed or trampled plants

Grazed and ungrazed *Tagetes patula* and *Bidens prestinaria* plants from the species association quadrats were collected, and the number of capitulas on each plant were counted. The data was used to find out whether any of the species produced more seeds/capitula when they were grazed. Mean number of capitula in grazed and ungrazed plants was put into a Bar diagram in EXEL.

2.3.7 Species association analysis

Areas where *Tagetes patula* was common were chosen as study sites. Quadrats of 2x2 metres were analysed (Figure 2.9) along the roadside, or from roadside towards more or less natural woodland, with 25 metres between each quadrat. The percentage cover of all plants in the quadrats was estimated. Altitude, latitude, longitude, aspect, and slope were recorded. Subjectively the disturbance of the squares was ranged in a scale between 0 (undisturbed) and 2 (heavily disturbed). Totally 26 quadrats were studied. The localities of the analysed quadrats are presented in Table 2.6, and illustrated in Figure 2.10.

Figure 2.9 Four 2 m long measuring sticks were used to make the quadrats. The local people were curious about what we were doing. Location outside Mandura village. Photo: Inger Nordal. Date: 10 Oct. 2005

The percentage cover data on species composition were transformed to ordinal transform values (OTV) (van der Maarel, 2005), and analyzed in a FORTRAN Computer Program TWINSPAN, TWo-way INdicator SPecies ANalysis, Version 1.0 (Hill 1979). TWINSPAN is a divisive polythetic method of vegetation classification. It classifies both samples and species. The resulting groups can be recognized as community types (Tesfaye *et al.* 2001). Four samples (14, 18, 21 and 22) became outliers, it was not possible to determine their exact position, and they were omitted because they disturbed the data. The number of pseudospecies cut levels was set to 3. The cut levels were 1, 3 and 6. The maximum number of species in the final tabulation was set to 40, in this way rare and common species were excluded from the final ordered two-way table. The default option was used in the rest of the analysis. Clustering analysis was done in PAST (Hammer *et al.* 2006).

Table 2.6 Species association analysis was carried out at three sites; Bulen, Dibate, and Mandura.

	Bulen	**Dibate**	**Mandura**
Latitude:	36 º 04.35` E	36 º 16.53` E	36 º 25.51` E
Longitude:	10 º 34.65` N	10 º 47.21` N	11 º 05.70` N
Altitude:	1725m	1575m	1425m
Releve:	R1-1 to R1-10	R2-1 to R2-5 R3-1 to R3-2	R4-1 to R4-9
Slope:	High, hill facing North	Moderate, facing North-West	Slightly, facing West
Disturbance:	All 2	All 2	From 0 to 2
Site description:	Partly covered by trees	More or less open roadsides	Partly covered by trees
Tagetes in gardens in village nearby:	No	Yes	Yes

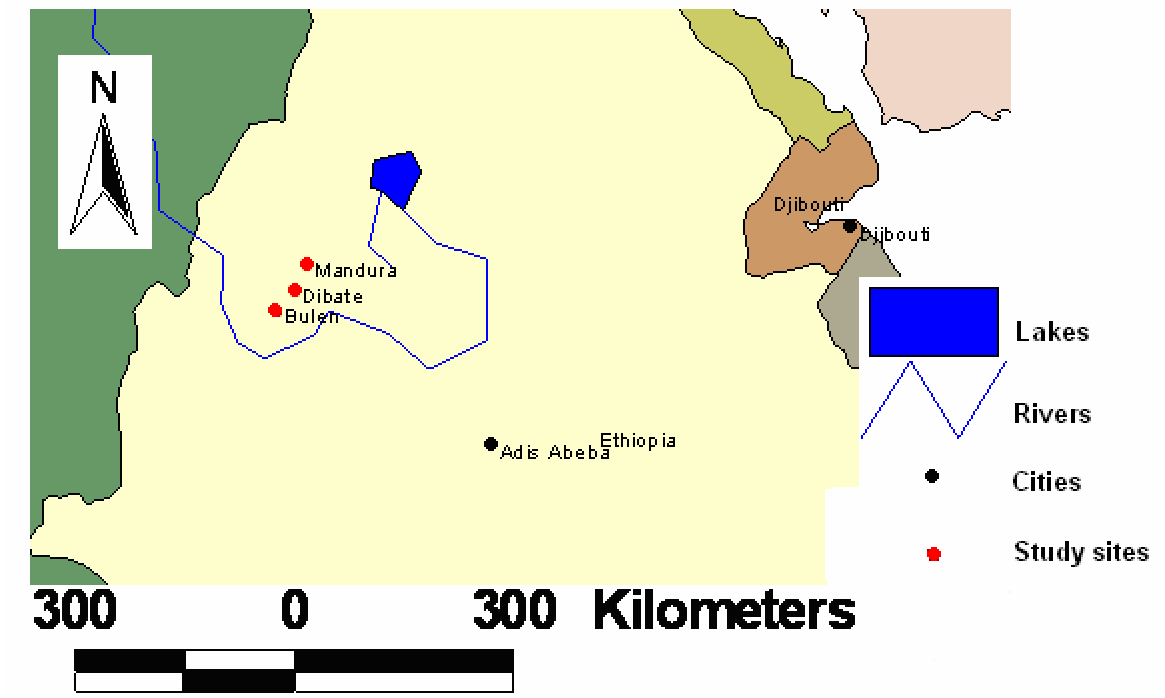


Figure 2.10 Study sites in Benshangul Gumuz National Regional State.

2.3.8 Soil seed bank

Field sampling

Soils from three sites with different dominance of *Tagetes patula* and *Bidens prestinaria* were collected. “Site 1”, the *Tagetes* dominated woodland (6 soil samples) was situated in Bulen, “Site 2”, the *Tagetes/Bidens* dominated hill (5 soil samples) was situated in Dibate, and “Site 3”, the *Bidens* dominated field (5 soil samples) was also situated in Dibate (Figure 2.10 & 2.11).

In each site, a different number of plots were randomly selected for species association analysis and soil sampling. The soil samples were taken underneath the target species. The surface of the soil was weeded, and the uppermost layer (ca. 0.3cm) removed. Two fairly close 10 x 10 cm blocks of soil, 4 cm deep were collected, and bulked. The soil samples were air-dried in the sun, and roots, rhizomes, tubers, undecomposed litter and plantlets were removed. The soil was stored in paper bags when dry, and transported to the University of Oslo.

a)

b)

c)

Figure 2.11 Soil samples were collected from three sites **a)** "Site 1" *Tagetes patula* dominated woodland, Bulen, **b)** "Site 2" *T. patula* & *B. prestinaria* dominated hill Dibate, and **c)** "Site 3" *B. prestinaria* dominated field Dibate. Photos A.B.S. 7 Oct. 2005.

Phytotron experiment

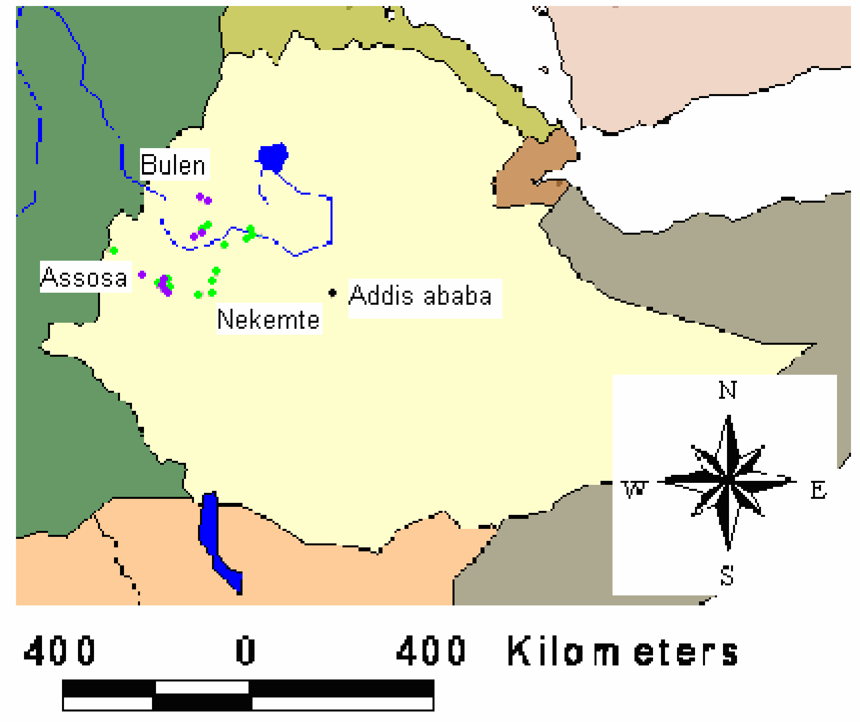
Two weeks after arrival in Oslo, the soil samples were weighted. Sixteen black trays (53 x 32 cm) with holes were filled to half their depth with a fresh sterile soil and perlite mix. This was compressed a bit, and then watered. The soil samples were distributed on top. A thin layer of sand was spread on top to avoid algae contamination. The trays were placed in a greenhouse, watered, and covered with plastic sheets to keep them moist. The greenhouse was set to day and night length of 12 hours at 25 ºC, and 20 ºC night, respectively. The soil samples were watered once every day. When germination began the plastic sheets were removed.

Emerging seedlings were recorded, tagged and potted on until they could be taxonomically identified. In addition one of each species was saved until flowering for pressing. After about five months the seedling emergence had stopped. The plants continued to grow vegetatively for a while, and in order to stress the plants to flower the watering was reduced to every third day. If still not flowering, one of each unknown or not yet flowering plant was transferred to a phytotron room for fully being able to control the light. The temperature was changed to 25 ºC during the daytime (12 h) and 15 ºC during the night (12 h). Mosses and ferns were not included in the study.

3. Results

3.1 Mapping distribution of escaped ornamentals in Benshangul Gumuz

Escaped *Tagetes patula* populations were found both South and North of the Blue Nile within the BGNRS (Figure 3.1). It was recorded in 24 localities as a cultivated ornamental, whereas in 9 localities the species was recorded as escaped. The area in which escapes have taken place is found in the Western parts of the total distribution, i.e. at lower altitudes.



Figur 3.1 Map of escaped and ornamental garden populations of *Tagetes patula*, in western Ethiopia. Green dots represent garden populations, while purple dots represent escaped populations.

Zinnia elegans was only observed escaped in one area (31.2 km East of Assosa), and is therefore not mapped.

3.2 Seed production

Flower heads of *Tagetes patula* and *Bidens prestinaria* were counted in each analysed plot ($4m^2$). In addition an average number of seeds per capitula were estimated based on 100 *T. patula* and 20 *B. prestinaria*. The seed production and the average number of capitula per plot are given in Table 3.1.

Table 3.1 Average seed production in *Tagetes patula* (n=100) and *Bidens prestinaria* (n=20), mean ± the S.D.

	Tagetes patula	***Bidens prestinaria***
# Capitula/Plot	4.0 ± 4.6	10.4 ± 24.0
Average # Seeds/Capitula	56.2 ± 8.7	43.8 ± 7.8
Average # Diaspore/Plot	Ca. 225	Ca. 455

Bidens prestinaria has less florets in total per capitula (ca. 44) than *Tagetes patula* (ca. 56). On the other hand, *B. prestinaria* has more flower heads per plot (ca. 10) than *T. patula* (ca. 4). Both species have a high seed production, *Bidens prestinaria*, however, have over twice as many diaspores per plot (ca. 455) compared to *T. patula* (ca. 225).

3.3 Information from local people

Tagetes patula

People from three different localities (two on the East-side of Assosa, and one near Mandura village) were interviewed about *Tagetes patula*. All of them explained that the species had been present for many years (“more than twenty”). In most places it was just called “the plant”, but in one location outside Dibate it was called the “*Derg weed*”. (The political Derg period took place from 1974 to 1991 (Gordon 2000), during which the plant most likely was introduced. In 1984 a large number of people were moved from areas in the North-East to the Western parts of Ethiopia to escape the famine).

It was, however, generally not characterized as a weed, partly because of its beauty. There was a general agreement that its main purpose was to be an ornamental for the local people. Negative impacts of the species were not mentioned, except for a minimum of weeding in domestic fields.

One man said that seeds had been spread from gardens to farmland due to garden cleaning where weeds, ashes and cattle faeces is removed and used as manure. Another man showed how the seeds easily could be spread manually. None of the interviewed people could confirm that it was eaten by animals. One young boy outside Mandura village made a necklace of the flower (see Figure 3.2).

Figure 3.2 Young boy outside Mandura village made a necklace out of *Tagetes patula*. The plant is not known to have any other purpose than being an ornamental in Benshangul-Gumuz. Photo: A.B.S., 10 Oct. 2005.

Bidens prestinaria

Three people (one Ado, one Oromo and one Amharic) were interviewed about the importance of *Bidens prestinaria* in the local environment. They explained that the species (popular name: Meskel daisy) was important in the production of honey. Meskel is a two day long festival celebrating the finding of the True Cross. (It is the most colourful festival after Timkat, Christ`s baptism). Bonfires are built topped by a cross decorated with the Meskel daisy (Tesfaye Awas, pers. comm.). Honey collected on a special day, the 1^{st} of October in the Ethiopian calendar (11^{th} of October Gregorian calendar) is believed to have a particular medicinal effect, and is not sold in stores, but kept for the family. Around this date the landscape is completely dominated by the Meskel daisy (see Figure 3.3) and the plants are accordingly the most important contributor to honey production. It is weeded from farm lands, for example the tef fields (*Eragrostis tef*, one of the most important crop species in the area), but is otherwise left undisturbed by man. It is grazed by domestic animals. The areas where it dominates are burnt annually. *Bidens prestinaria* is endemic for Ethiopia and Sudan, and has “always” been there.

Figure 3.3 *Bidens prestinaria* dominated vegetation. Photo: A.B.S., 7 Oct. 2005 Missionary road outside Dibate village.

3.4 Fire simulation experiment

The germination of the pre-treated diaspores of *Tagetes patula*, *Zinnia elegans* and *Bidens prestinaria* (control, 60ºC/1 minute, 60ºC/5 minute, 90ºC/1 minute, 90ºC/5 minute, 120ºC/1 minute and 120ºC/5 minute) was followed for 18 weeks. Within this time seeds of the three plant species in the experiment had germinated (Figure 3.4).

Tagetes patula had the longest diaspores of the three species. *Zinnia elegans* had the heaviest diaspores, and were intermediate in size (Table 3.2). *Tagetes patula* had a very fast germination response, as all seeds had germinated within the first week (independent of treatment). In contrast *Z. elegans* required 13 weeks to reach full germination. *Bidens prestinaria* had the lightest, and shortest diaspores, but needed the longest time, of the three species, to germinate (18 weeks).

Table 3.2 Description of the diaspore characteristics of the three study species (mean ± S.D., n =10 per species) * Experiment finished

Plant species	**Diaspore length (mm)**	**Diaspore weight (mg)**	**First day of germination**	**Duration of germination**
Tagetes patula	10.4± 0.6	1.9± 0.4	2. day	6 days
Zinnia elegans	6.3± 1.0	4.9± 0.8	3. day	91 days
Bidens prestinaria	6.0± 0.8	2.6 ± 2.4	7. day	126* days

Tagetes patula	*Bidens prestinaria*	*Zinnia elegans*

Figure 3.4 Germinating seeds from the fire simulation experiments. Photos: A.B.S., December 2005 in the Phytotron at UoO.

The germination response for the three studied species is shown in Figure 3.5. The *Tagetes patula* seeds started germinating on the second day after sowing. The germination rate for seeds exposed to higher temperatures was slightly lower compared to the control (see Figure 3.5**a**) where the three last bars belong to treatment 120ºC/1 minute).

Zinnia elegans started germinating after three days. The last germination was registered 91 days after sowing. Under the different temperature and duration treatments the seeds germinated approximately evenly over the germination period (see Figure 3.5**b**).

Bidens prestinaria started germinating after seven days. The last germination was registered 126 days after sowing, but ungerminated seeds were still present. Under the different temperature and duration treatments the seeds germinated approximately evenly over the germination period (see Figure 3.5**c**).

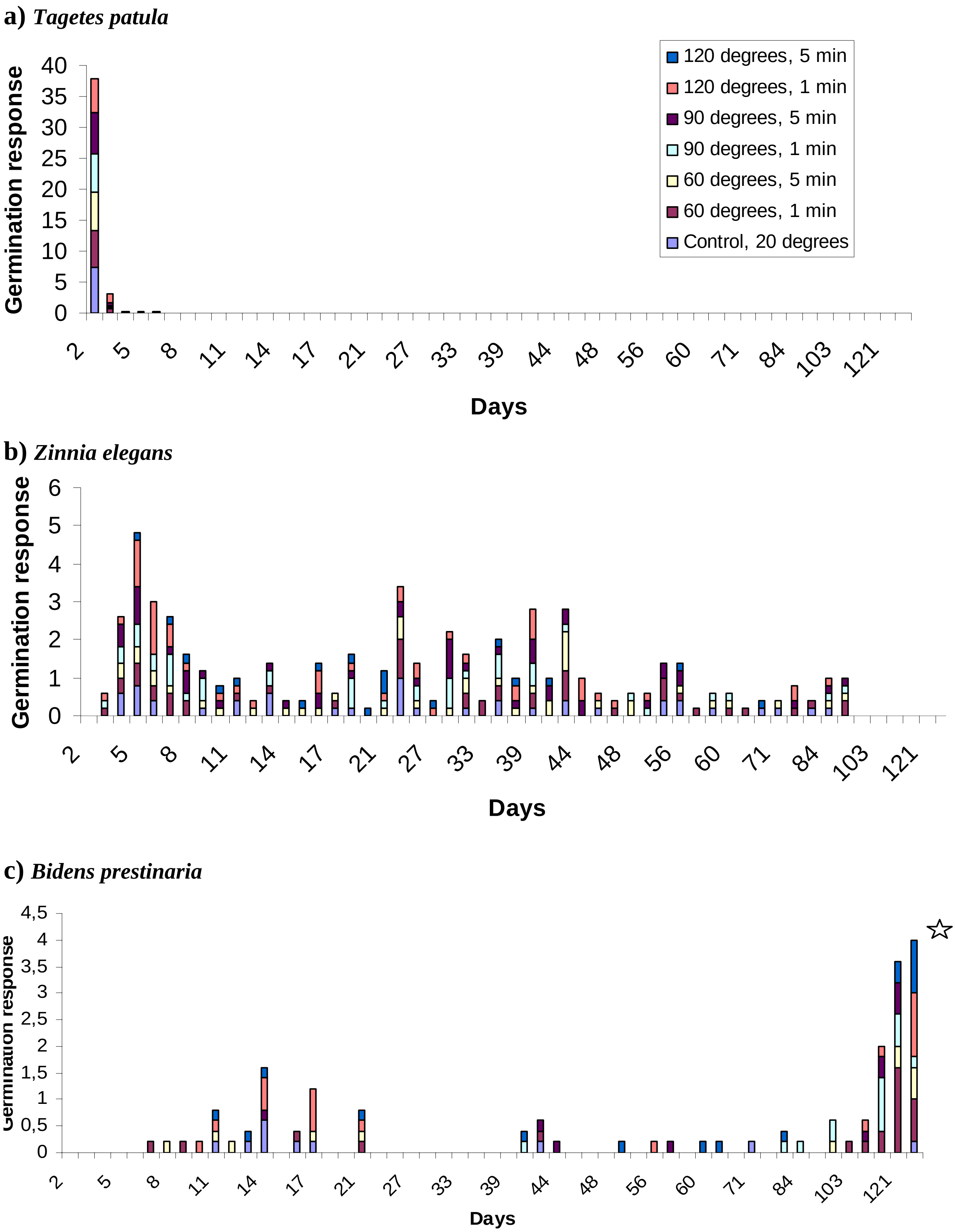


Figure 3.5 Germination response over an 18 week long germination period for **a)** *Tagetes patula*, **b)** *Zinnia elegans*, and **c)** *Bidens prestinaria* cypselas exposed to 7 different heat shock treatments. Average values where n = 50 are used. The star in c) indicates that the experiment finished due to time limitation.

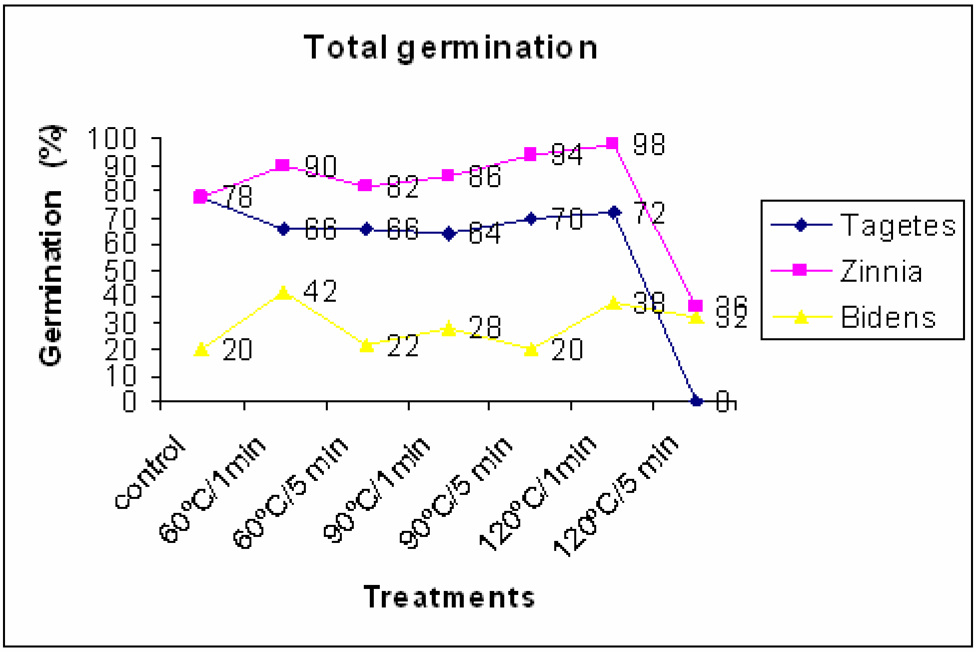


Figure 3.6 Germination rate for the three study species *Tagetes patula*, *Zinnia elegans* and *Bidens prestinaria* exposed to different heat shock treatments.

Mean total germination frequency in *Tagetes patula* for all the treatments combined was 59% (± S.D. 17%). The total germination frequency was highest in the control treatment (78%) and stayed rather high up to the 120ºC/1 minute treatment. The diaspores in the 120ºC/5 minute treatment died, and no germination was obtained (Figure 3.6).

Mean total germination frequency in *Zinnia elegans* for all the treatments combined was 81% (± S.D. 13%), with highest germination frequency in the 120ºC/1 minute treatment (98%). *Zinnia elegans* had the highest germination frequency in all treatments compared to the other species. The control treatment (78%) gave the lowest germination frequency of all treatments except the 120ºC/5 minute treatment (36%). The diaspores tolerated the highest heat shock treatment, and the germination frequency increased with higher temperatures compared to the control treatment.

Mean total germination frequency in *Bidens prestinaria* for all the treatments combined was 29% (± S.D. 7%), the lowest frequency of the three species. *Bidens prestinaria* showed an increased germination frequency in the intermediate and higher heat treatments compared to the control treatment (20%). The 60ºC/1 minute treatment gave the highest germination frequency (42%).

Table 3.3 Tukey`s pairwise comparison: Q / p(same) from PAST for **a)** *Tagetes patula*, **b)** *Zinnia elegans* and **c)** *Bidens prestinaria*. The significant values are marked with yellow.

a)

Tagetes patula	Control	60-1	60-5	90-1	90-5	120-1	120-5
Control	0	0,5951	0,5951	0,4178	0,9025	0,9741	0,000145
60-1	2,464	0	1	0,9999	0,9969	0,9741	0,000145
60-5	2,464	0	0	0,9999	0,9969	0,9741	0,000145
90-1	2,875	0,4107	0,4107	0	0,9741	0,9025	0,000145
90-5	1,643	0,8214	0,8214	1,232	0	0,9999	0,000145
120-1	1,232	1,232	1,232	1,643	0,4107	0	0,000145
120-5	16,02	13,55	13,55	13,14	14,37	14,79	0

b)

Zinnia elegans	Control	60-1	60-5	90-1	90-5	120-1	120-5
Control	0	0,7347	0,9986	0,9477	0,4263	0,1897	0,0003623
60-1	2,141	0	0,9477	0,9986	0,9986	0,9477	0,000147
60-5	0,7135	1,427	0	0,9986	0,7347	0,4263	0,0001914
90-1	1,427	0,7135	0,7135	0	0,9477	0,7347	0,000155
90-5	2,854	0,7135	2,141	1,427	0	0,9986	0,0001454
120-1	3,568	1,427	2,854	2,141	0,7135	0	0,0001451
120-5	7,492	9,632	8,205	8,919	10,35	11,06	0

c)

Bidens prestinaria	Control	60-1	60-5	90-1	90-5	120-1	120-5
Control	0	0,4853	1	0,9917	1	0,7014	0,9382
60-1	2,714	0	0,5937	0,88	0,4853	0,9998	0,9739
60-5	0,2467	2,467	0	0,9983	1	0,7995	0,9739
90-1	0,9869	1,727	0,7402	0	0,9917	0,9739	0,9998
90-5	0	2,714	0,2467	0,9869	0	0,7014	0,9382
120-1	2,22	0,4934	1,974	1,234	2,22	0	0,9983
120-5	1,48	1,234	1,234	0,4934	1,48	0,7402	0

One Way Anova statistics was used on the data to find out if there were significant differences in germination frequency in the different temperature treatments. The total p-value between groups for *T. patula*, was 6.136E-11, there is therefore a significant difference between treatments to a confidence interval of at least 95%. The p-value between groups for *Z. elegans* was 3.09E-7, also significant, whereas the p-value for *B. prestinaria* was 0.3381, thus not significant.

Table 3.3 shows that it is the 120ºC for 5 minutes treatment that gives the significant reduction in germination for both *Tagetes patula* and *Zinnia elegans*. *Bidens prestinaria*, however, has no treatments with significant lower germination, in the pairwise comparison, and is therefore (most likely) not affected by the increasing heat treatments, including the 120ºC for 5 minutes treatment.

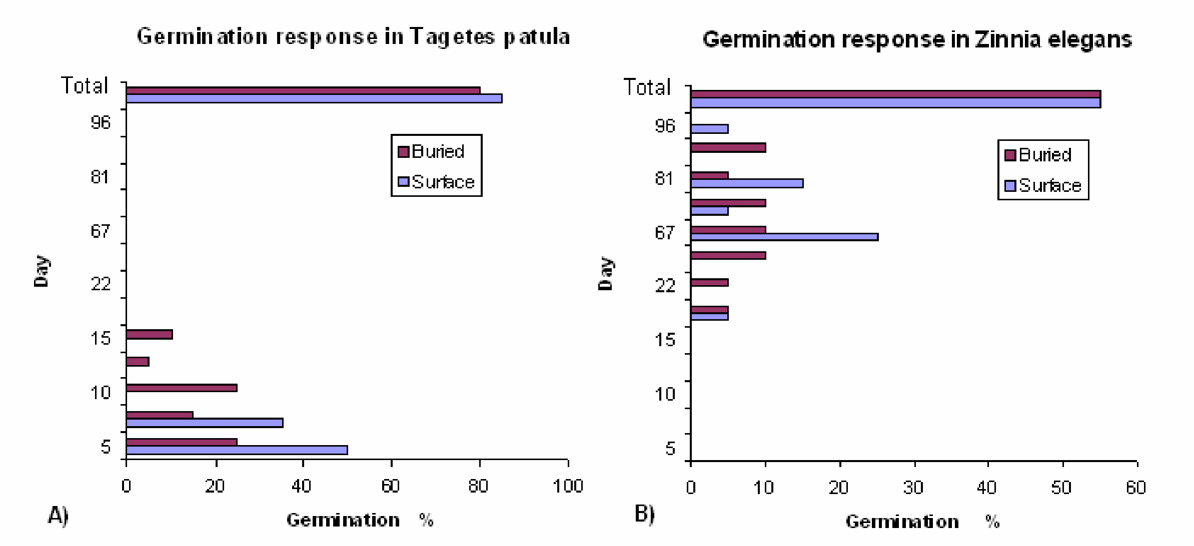
3.5 Germination experiments

To test whether the diaspores were dependent on light or depth to germinate, diaspores of *Tagetes patula*, *Zinnia elegans* and *Bidens prestinaria* were sown on the surface (Treatment 1), or under 1 cm soil (Treatment 2).

Tagetes patula seeds started germination 5 days after sowing. All seeds had germinated after 15 days (Figure 3.7**A**). Diaspores sown on top of the soil germinated before diaspores planted under 1 cm soil. The average total germination frequency for diaspores planted on top of the soil was 85%, while the germination frequency for diaspores planted under a 1 cm layer of soil was 80%.

Zinnia elegans seeds started germination 18 days after sowing. All seeds had germinated after 95 days (Figure 3.7**B**). The soil layer had no observable effect on the time required to germinate. Germination frequency for both diaspore treatments were on average 55%.

Bidens prestinaria seeds never germinated during the period of observation (ca.150 days), and is therefore not included in the study.



Figur 3.7 Germination response in *Tagetes patula* and *Zinnia elegans* exposed to two different treatments: “Buried”, diaspores sown under 1 cm soil, “Surface”, diaspores sown at the surface of the soil.

Both treatments of *Tagetes patula* diaspores gave similar growth curves (Figure 3.8). The plants grew slowly for about 35 days, after which growth increased rapidly. Most plants started flowering after 140 days. Diaspores sown on top of the soil (Treatment 1) got a slightly higher number of flower heads than the plants from buried diaspores (Treatment 2). The average plant height when flowering was about the same in the two treatments (ca. 95cm), see Table 3.4.

Table 3.4 Height obtained for *Tagetes patula* exposed to one of the two treatments. Values are the averages of the germinated seeds, n=10 with two repetitions in each treatment ± S.D.

	Treatment 1 Seeds sown on soil surface	**Treatment 2** Seeds buried under 1cm soil
Average height	96.3 ± 5.9	95.7 ± 7.0
Average # of capitula	19.3 ± 3.3	16.9 ± 3.8

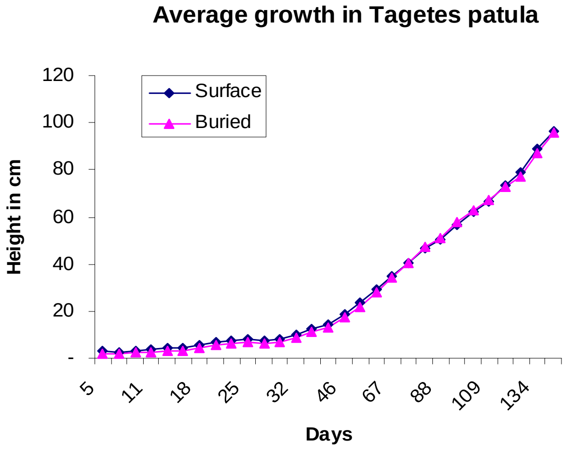


Figure 3.8 Average growth in cm for *Tagetes patula* sown on the soil surface, or buried under 1 cm soil.

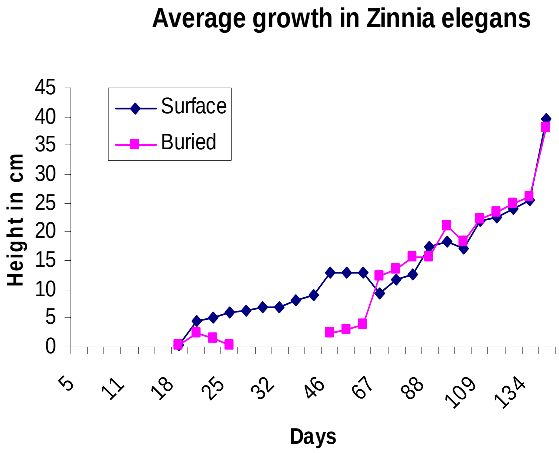


Figure 3.9 Average growth in cm for *Zinnia elegans* sown on the soil surface, or buried under 1 cm soil.

Zinnia elegans showed a more irregular growth curve than *Tagetes patula*. No clear trends for either of the treatments was, however, observed (Figure 3.9). Several plants died during the experiment for unknown reasons. This may have affected the results. The first plant started flowering after 145 days. Only one plant was flowering before the experiment ended, after about 150 days. This plant was ca. 70 cm when flowering.

3.6 Flower production in grazed or trampled plants

Disturbance (in form of animals eating top shoots) was observed to stimulate growth from the side shoots of both *Tagetes patula* and *Bidens prestinaria* plants in the field. In ten random quadrats with both grazed/trampled and "normal" specimens observed, 25.4 % of the *Bidens prestinaria* plants were grazed/trampled, and 15.5 % of the *Tagetes patula* plants were grazed/trampled.

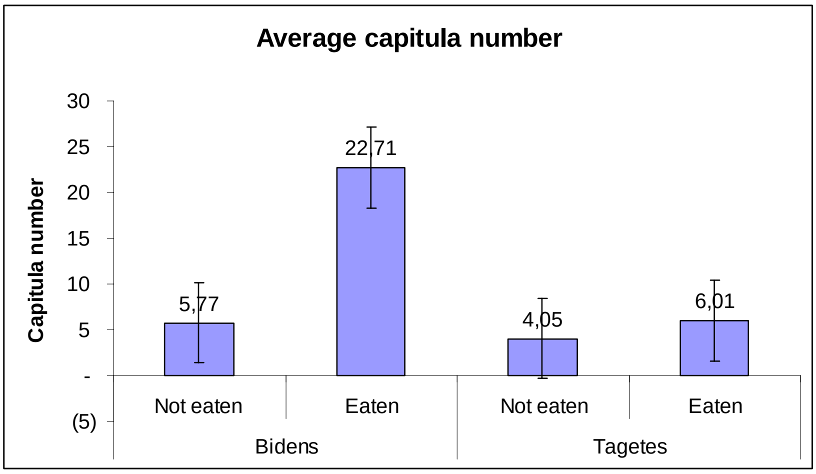


Figure 3.10 Average number of capitula per plant (grazed/trampled = eaten and "normal" = Not eaten) in *Bidens prestinaria* (n = 378) and *Tagetes patula* (n= 485) ± the standard error.

In both ungrazed and grazed specimens *Bidens prestinaria* have a higher capitula number than *Tagetes patula* (Figure 3.10). The grazed *B. prestinaria* shows a significantly larger capitula production per plant (ca. 22) than the specimens that were left undisturbed (ca. 5). Grazed or trampled *T. patula* also seem to produce a higher number of capitulas (ca. 6 versus ca. 4).

Both in the field and in the Phytotron it was noticed that damaged, broken plants compensated by growing extra shoots. Side shoot formation was stimulated, even when the main stem was broken only a few cm above the ground level. This tendency was observed in both *Bidens prestinaria* and *Tagetes patula*, it was, however, not found in *Zinnia elegans* plants (which regularly only produce one flower head per plant).

3.7 Species association analysis

A total of 132 plant species were found in the 26 quadrats analysed. The species belonged to 40 different plant families. The most species rich families were Poaceae (25 species), Asteraceae (15) and Fabaceae (12). Three taxa were sterile and impossible to identify, and 12 could only be referred to family or genus. For a complete species list, see Appendix A.

The data matrix was analysed in TWINSPAN. The ordered two-way table from TWINSPAN (Table 3.5) shows the plant associations (communities) revealed by the analysis. The table has been ordered to exhibit the relation between the species and the samples as clearly as possible. The species on the top are more abundant on the left side of the primary division than on the right side. The species on the bottom are more abundant on the right side of the primary division than on the left side. The species in the middle are somewhat indifferent, occurring widely on both sides. These species are generally not interesting for the study of the focal species (*Tagetes patula* and *Bidens prestinaria* (indifferent, common species), marked green in Table 3.5).

Table 3.5 The ordered two-way table from TWINSPAN (Hill 1979) shows the plant associations where *Tagetes patula* most likely can be found. Species names are shown at the left, sample numbers along the top, and the classifications of species and samples are indicated along the right and bottom margins. The main divisions of the species are indicated by a horizontal line, the same for the samples is indicated by vertical lines. Values indicate a scale of abundance, with absence represented by -.

	Species	**Plot**		
		1	212112111122	
		5680271349	690233156745	
113	Paspalum scrobiculatum	22-121-111	---2--------	000011
140	Sporobolus piliferus	322211-11-	-2-----2----	000011
156	Ziziphus abyssinica	---133--3-	--2---------	000011
25	Asterolinon adoense	122122--2-	------------	000100
135	Spermacose chaetocephala	2122111---	---1--------	000100
80	Guizotia shimperii	333233-333	---1--------	000110
30	Brachiaria brizantha	1-1111111-	------------	000111
34	Centella asiatica	2333332323	------------	000111
88	Justicia ladanoides	-11-111-11	---1--------	000111
16	Aneilema hirtum	--1-112111	-1----------	00100
43	Commelina subundulata	-11-122111	-1-1--1-----	00110
45	Crotalaria macrostachys	----2-332-	------3-----	00111
74	Galinsoga quadriradiata	----1-2122	----------11	00111
82	Hygrophilia attriculata	2112--232-	222--1--1-1-	0100
94	Leucus martinicensis	-111-1212-	1--1--112---	0100
117	Phyllanthus pseudoniruri	11-1211122	--11--1111-1	0100
136	Spermacose sphaerostigma	122-221121	3-1221111-11	0100
119	Plectranthus 1427	---1112111	---22-11----	0101
64	Eragrostis schweinfurthii	-2-1-1----	--23--------	0110
35	Chloris pycnothrix	2-12------	-2----1-3---	0111
12	Ageratum conyzoides	111-11-321	21-2-1212--1	1000
28	Bidens prestinaria	21--112141	-1-2-32323--	1000
58	Digitaria ternata	2221111211	-223--131---	10010
146	Trifolium rueppellianum	1221231211	1322--222---	10010
31	Bracharia semiundulata	-11211--11	3-22--22-1--	10011
130	Setaria pumila	1-111-1111	2321-12121--	101
86	Jasminum grandiflorum	----------	------23-3--	110000
122	Rhyncosia nyasica	----------	-111--211311	110001
5	Acacia polystachyon	----------	-1-3132233--	110010
62	Eleusine africana	-------1--	2332--113---	110010
105	Ocimum trichodon	----------	--212-1321--	110010
111	Panicum atrosanguineum	1---------	-121---21---	110010
115	Pennisetum polystachion	----------	3-22122-2---	110011
26	Bidens biternata	--------1-	211222222221	110110
72	Flueggia virosa	----------	2-23323---33	110110
120	Polygala persicariifolia	--------1-	-111111--211	110110
143	Tagetes patula	1---13222-	3123333333--	110111
106	Oplismenus burmannii	-------2--	-23-32----33	11100
69	Ficus sycomorus	----------	------3---33	111011
4	Acacia hecatophylla	-----2----	----------33	1111
		0000000000	111111111111	
		0000001111	000000000011	
		011111	0000001111	
		00011	011111	
			00111	
	Association:	1	2	

Particularly three species, *Asterolinon adoense*, *Centella asiatica*, and *Guizotia schimperi* (marked with a blue colour in Table 3.5) demarcate the group of samples to the left of the primary division ("Association 1"), and are indicative of conditions in which other species of this association are likely to be found. The species marked in yellow (*Bidens biternata* and *Rhyncosia nyasica*) are only found in "Association 2" right of the primary division, and act as character species for this association.

The species in "Assiciation 1" are mainly herbs, only *Ziziphus abyssinica* is shrubby. In "Assiciation 2" three trees are found *Acacia hecatophylla*, *A. polystachyon* and *Ficus sycomorus* in addition to one shrubby species, *Flueggia virosa.* Of the species characterising "Assosiation 1" several are connected to open habitats (e.g. *Sporobulus piliferus*, *Guisotia shimperi,* and *Brachiaria brizanta*). A species like *Oplismenus burmannii* mainly found in "Association 2" is known to prefer shady, often moist places (Edwards *et al.* (1995, 1997 and 2000), Hedberg & Edwards (1989), Hedberg *et al.* (2003), Mesfin Tadesse (2004) and Pillips, (1995). Weedy species are found in both associations; *Guizotia schimperi* and *Galinsoga quadriradiata* in "Association 1", and the grasses *Eleusine africana*, *Panicum atrosanguineum* and *Penisetum polystachyon* in "Association 2".

It is important to note that only two plots (24 & 25) were recorded with disturbance = 0. They are linked to "Association 2". Only one, of the mentioned weeds (*Galinsoga quadriradiata*) is found in these plots. *Tagetes patula* is not found in these two plots.

The data were further analysed by different cluster analyses, using Euclidean, Jaccard, Correlation and Bray Curtis similarity measures, all giving the same tree topology. The presented tree (Figure 3.11) is based on Bray Curtis similarity. The tree reveals two main groups, one consisting of the undisturbed plots 24 and 25, the other main group comprising the rest. The second cluster subdivided into two subclusters, the first consisting of the "Association 1" plots, and the second of the "Association 2" plots, except for plots 24 and 25.

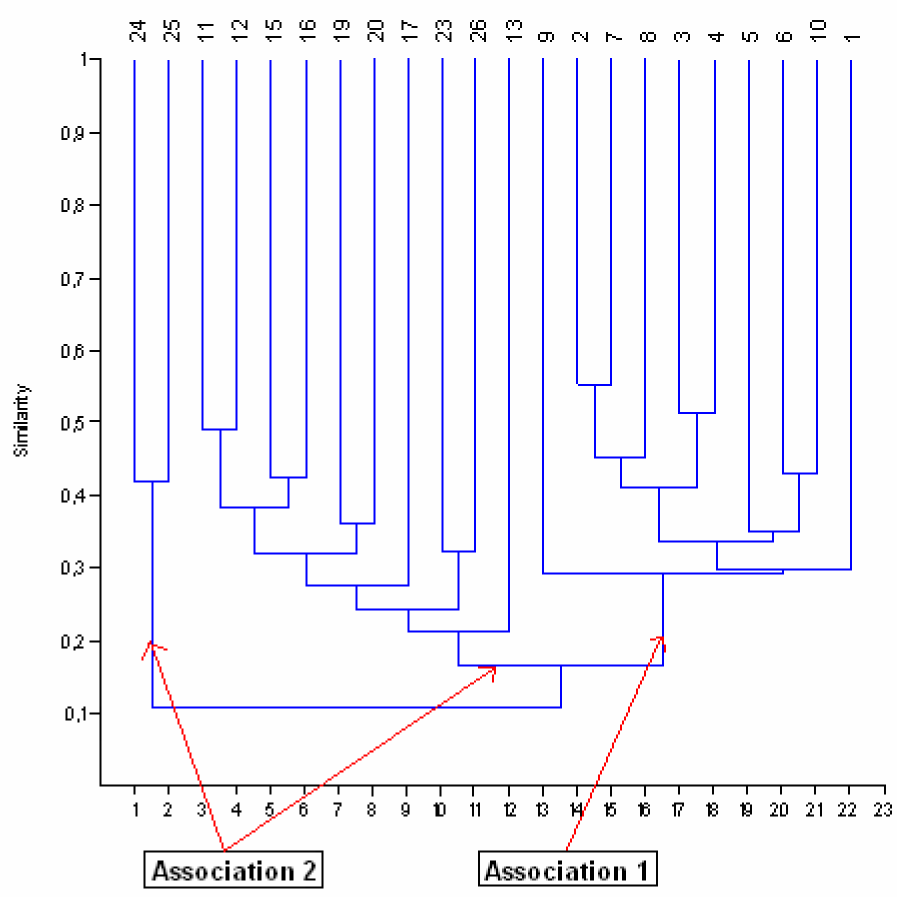


Figure 3.11 Cluster analyses of the plots using Bray Curtis similarity. The main division is separating the two undisturbed 24 and 25 from the rest. "Association 1" and "Association 2" refer to the main division of the TWINSPAN analysis.

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3.8 Soil seed bank

The species composition in the soil seed banks is summarised in Table 3.6. The soil samples were collected from three sites: Six from “Site 1” *Tagetes* dominated woodland, five from “Site 2” *Tagetes*/*Bidens* dominated hill, and five from “Site 3” *Bidens* dominated grazing field. A total of 44 species and 1204 seedlings germinated from the soil samples. Of these, 800 were recorded from seed banks in “Site 1”, 193 seedlings were recorded from seed banks in “Site 2”, and 211 seedlings were recorded from seed banks in “Site 3”. Both of the target species, *Tagetes patula* and *Bidens prestinaria* appeared in the seed bank, the former with 10 seedlings in total (7 in “Site 1”, and 3 in “Site 2”), and the latter with 16 seedlings in total (14 in “Site 1”, 1 in “Site 2”, and 1 in “Site 3”).

Three different growth forms appeared in the seed bank: monocots (grasses), herbaceous dicots and trees. Spore plants, such as ferns and mosses were observed, but not taken into account. Herbaceous dicots, trees and grasses were represented in the soil seed banks from all three sites, but trees were less abundant in “Site 2” (1 species), than in “Site 1” (4 species). Monocots were generally less abundant than dicots, 7.5% versus 92.5% respectively. Annual species dominated the soil seed bank, reflecting a degree of disturbance in all groups. Most plants were dependent on seeds for reproduction, whereas the dominating perennial species *Centella asiatica* and *Acmella caulirhiza* had the ability to grow clonally in addition to the seed reproduction.

A total of 18 families and 40 genera were represented in the seed banks of which Asteraceae occurred with the highest number of species (11). The families Moraceae, Boraginaceae, Tiliaceae, Cucurbitaceae, Rubiaceae, Commelinaceae, Lobeliaceae, Apiaceae, Lamiaceae, Malvaceae, and Primulaceae were represented with only one species each. Apiaceae, Asteraceae, Euphorbiaceae, Lobeliaceae, Poaceae, and Rubiaceae constituted the highest abundance of seedlings in the seed bank with together 87%. Apiaceae alone represented 50.5% of all the seedlings. The remaining families Solanaceae, Fabaceae, Acanthaceae and Amaranthaceae were represented at lower abundances in the seed bank.

The species richness in the seed bank was highest in “Site 1” with 37 species and lowest in “Site 2” with only 25 species. The total number of species in a single plot varied from 6 species in “Site 3” plot 5, to 19 species in “Site 1” plot 1. “Site 1”, plot 1 and “Site 3”, plot 1 had most species, while “Site 1” plot 2, “Site 2” plot 1 and “Site 3” plot 5 had the lowest number of species (Figure 3.13, s. 61).

The total number of seedlings also showed large variation among plots, with the highest number of seedlings in “Site 1”, plot 5 (229 seedlings), while “Site 3”, plot 5 had the lowest number (16) of seedlings. Plot 1, plot 2 and plot 5 in “Site 1” generally have a higher number of seedlings than the rest of the plots (Figure 3.14, s. 61).

Seedling numbers were converted to seedlings per 1 kg soil. The seedling density was lowest in “Site 2” with 74.8 seedlings per 1 kg soil, and highest in “Site 1”, with 157 seedlings per 1 kg soil (Figure 3.15, s. 61).

The seed banks from different sites were characterized by different dominant species. Altogether 26 of the 44 identified species were unique for one of the sites. Fourteen species were unique for “Site 1”, whereas “Site 2” only had 3 unique species. “Site 3” had 9 unique species. Non-unique species were observed in all three sites.

The vast majority (69%) of the seedlings in the soil seed banks were represented by *Acmella caulirhiza*, *Ageratum conyzoides*, *Centella asiatica*, *Euphorbia hirta* and *Lobelia* sp. Plots in “Site 1” were dominated by *Centella asiatica* (highest seedling frequency 608), *Dicrocephala integrifolia, Vernonia theophrastifolia*, and an unidentified grass (Poaceae C). *Vernonia theophrastifolia* was also found in plots from “Site 2”, but *Chloris virgata*, *Ageratum conyzoides* and *Acmella caulirhiza* were more dominating. *Ageratum conyzoides*, *Euphorbia hirta*, *Lobelia* sp., and *Oldenlandia corymbosa* were common in plots from “Site 3” (Figure 3.12, s. 60).

Some species were found in the soil seed banks, and not in the vegetation analysis (Table 3.6). These are: *Achyranthes aspera, Celosia trigyna, Hibiscus calyphyllus, Phyllanthus ovalifolius, Solanum alatum, Cordia africana*, and *Eragrostis tef* . *Oldenlandia corymbosa, Senecio sp., Vernonia theophrastifolia*, and *Chloris virgata* are, in addition to only being found in soil seed banks, dominating species with a high number of seedlings.
Other species were only observed in the vegetation analysed plots, and not in the soil seed banks. The most dominating of these were (see Appendix A): *Lannea fruticosa, Crassocephalum rubens, Galinsoga quadriradiata, Bidens biternata, Lobelia inconspicua, Commelina subulata, Aneilema hirtum, Senna obtusifolia, Leonotis osymifolia, Plectranthus* sp., *Ocimum trichodon, Polygala persicariifolia, Spermacose sphaerostigma*, and *Kohautia tenuis*.

Table 3.6 Number of seedlings pr. species in the soil seed banks of the tree different groups. The most dominating species are marked bold and blue. The species that occurred only in the seed bank are marked bold and red. Both dominating and "new" species are marked bold and pulple. The target species are marked yellow.

Group:	1. Tagetes dominated woodland						2. Tagetes/Bidens dominated hill					3. Bidens dominated field				
Plots	**1**	**2**	**3**	**4**	**5**	**6**	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**
Herbaceous dicots:																
***Achyranthes aspera* L.**							1	1		1						
***Acmella caulirhiza* Del.**	19			2	1		1	1	7	1	1				1	
***Ageratum conyzoides* L.**	1						2	6	4	9	1	1	7	2	4	
Asterolinon adoense O. Kunze															3	3
Bidens pilosa L.						2						4				
***Bidens prestinaria* (Sch. Bip.) Cuf.**					1	13		1				1				
***Celosia trigyna* L.**													1			
***Centella asiatica* (L.) Urban in Mart.**	125	173	11	78	210			10			1					
Commelina imberis Ehrens. Ex. Hassk										1						
***Dicrocephala integrifolia* (L. F.) O. Kuntze**	2	11	3	3	1		1									
Ethulia gracilis Del.						1				1						
***Euphorbia hirta* L.**			3					2	6				10	7	3	1
Guizotia schimperi Sch. Bip. Ex Walp.	1	2	1	1					1							
***Hibiscus calyphyllus* Cavan**					1							1		3		
Hygrophila attriculata (Schumach.) Heine			1		1											
Justicia sp.		1														
Leucus martinicensis (Jacq.) R. Br.					2					3						
***Lobelia* sp.**	28		1		1				23	2	1		22	18	17	8
***Oldenlandia corymbosa* L.**			5	1			1		5	2		2	6	2	3	
***Phyllanthus ovalifolius* Forssk.**												1				
Phyllanthus pseudoniruri Muell. Arg.													1			
***Senecio* sp.**						3		1	3	1				2	2	1
***Solanum alatum* Moench.**											1	1				
***Tagetes patula* L.**		2				5				3						
Triumfetta rhomboidea Jacq.												1				
Trifolium rueppellianum Fresen					1					1						
***Vernonia theophrastifolia* Schweinf. Ex Oliv. & Hiern**	2	1	3			5	8	1		1	1	1	2	3		

Unknown, or died before classification (herbs)	9	13	6	4	8	4	6	3	11	4	3	15	9	9	7	3
Trees:																
Acacia polystachya A. Cunn ex. Benth.												1				
***Cordia africana* Lam.**						1										
Ficus sycomorus L.												2				
Unknown (trees)					1	2		1								
Grasses:																
***Arthraxon micans* (Nees) Hochst.**	1		1			3		2	5					2	1	
Brachiaria brizantha (A. Rich) Stapf.	1														1	
***Chloris virgata* Sw.**	1		1		1			8	4	1	5		2		1	
Digitaria ternata (A. Rich.) Stapf.	1															
***Eragrostis tef* (Zucc.) Trotter**			1													
Panicum sp.	1															
Pennisetum petiolare (Hochst.) Chiov.	1			1												
Unknown grass seedlings	2	1	3	3			1	14	2		5	11			2	
Number of seedlings:	195	204	40	93	229	39	21	51	71	31	19	42	60	48	45	16
Seedlings pr kg soil:	227	263	38	86	259	71	35	69	175	69	26	83	111	140	145	58
Number of species:	19	8	15	11	13	11	8	14	12	14	10	17	11	11	13	6

a)

b)

c)

Figure 3.12 The most dominating species in the three seed banks: **a)** "Site 1"-*Tagetes* dominated woodland: *Centella asiatica* (left) and *Dicrocephala integrifolia* (right), **b)** "Site 2" – *Tagetes/Bidens* dominated field: *Ageratum conyzoides* (left) and *Acmella caulirhiza* (right), and **c)** "Site 3" – *Bidens* dominated hill: *Euphorbia hirta* (left) and *Lobelia* spp. (right) Photos: A.B.S.

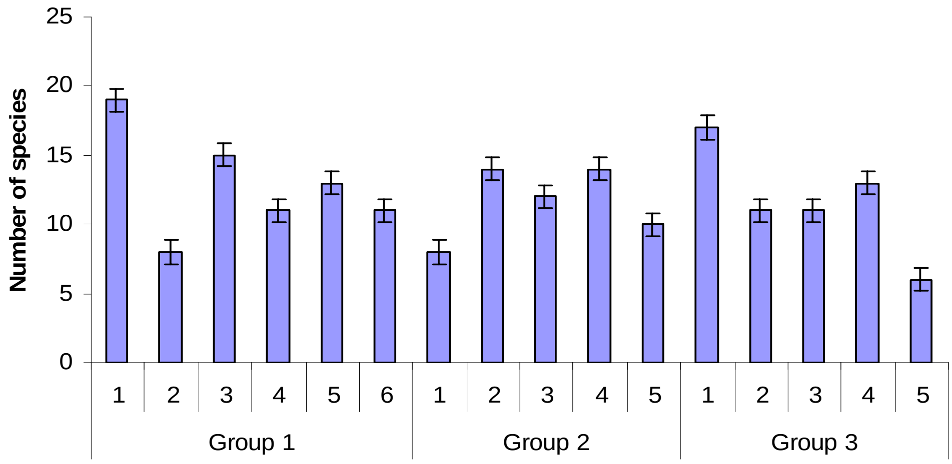


Figure 3.13 Number of species pr. plot in the three sites/groups ± s.e. (Group = Site).

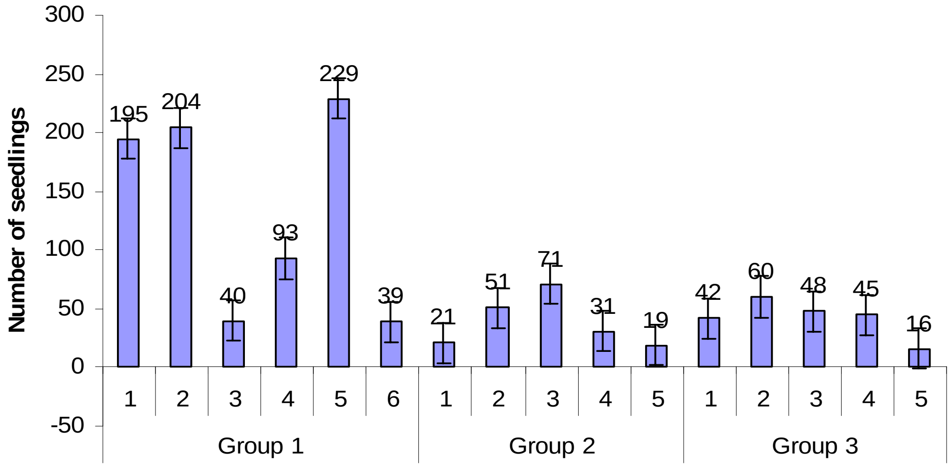
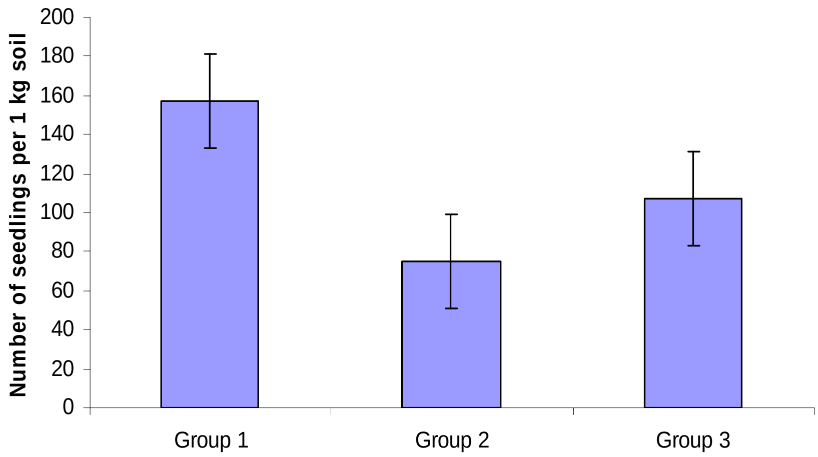


Figure 3.14 Total number of seedlings in the soil seed bank (exact numbers at the top of the bars) in the different plots, ± s.e. (Group = Site).



Figur 3.15 Seedlings per 1 kg soil ± s.e. for each of the different groups (Group = Site).

4. Discussion

4.1 Does the introduced ornamental species *Tagetes patula* and *Zinnia elegans* escape from gardens?

The map, showing cultivated and escaped *T. patula* populations, clearly documents that the species has escaped over fairly large areas in Benshangul Gumuz National Regional State (BGNRS). The species has been grown in gardens in the area for more than 20 years, and since Tesfaye Awas (pers.comm.) first observed escapes in 2001, the invasion in areas outside gardens has apparently increased. It has repeatedly been shown that populations may increase according to a sigmoid curve after escape/invasion (Graham, 1939). Initially, the population grows more slowly in absolute size, reaches a maximum rate of increase near the middle of the curve, and grows slowly again as it approaches the asymptote of maximal density. Being recently introduced, *Tagetes patula*, as an exotic colonizer, might be situated in the first slowly increasing phase of the curve, in which its impact is rather insignificant. The exponential part of the curve might not have been reached, but if/when such a phase is obtained, the impact on other species might increase considerably, and *T. patula* might create a biodiversity problem, even to endemics and other rarities in the area.

The escaped populations are found in altitudes from ca. 1100 m to ca. 1900 m. Garden populations are found in altitudes up to 2300 m. *Tagetes patula* appears, accordingly not to escape at high altitudes where low temperatures possibly restricts its competitive potential. In addition, there might be less available niches at higher altitudes because of the higher density of people. Another reason for escapes, only at lower altitudes, might be that the species has existed in cultivation for a longer period here, and accordingly has had more time to escape in these regions.

For comparison, *Zinnia elegans*, which was frequently observed in cultivation in BGNRS, although not as frequent as *Tagetes patula*, was only found escaped in one locality in the surveyed area (visualized in Figure 3.1). *Zinnia elegans* was probably introduced during the resettlement program when people were moved from areas in the North to Western parts of Ethiopia in 1984, and has therefore been introduced more or less at the same time as *T. patula* (see p. 40). *Zinnia elegans* is accordingly not as invasive as *T. patula,* thus representing a lower potential threat to the local plant diversity.

4.2 Which habitat types are vulnerable to invasion of *Tagetes patula*?

The species association analyses revealed that *Tagetes patula* was mainly found in “Association 2” characterised by including tree species and also species demanding some shade and moisture. It was less frequent in “Association 1” which, as judged from the species composition represents open and more arid habitat types. Both associations include weedy species, indicating that a certain amount of disturbance has taken place. The quadrats exposed to a minimum of disturbance, however, did not house *Tagetes patula*. The species appears to depend on some disturbance, but avoid the driest and most sun exposed areas. The seeds may also be dependent on disturbance from humans, human activities and animals, to spread and germinate. Pristine woodlands are probably not in danger of severe invasions, but woodland margins might probably be invasible.

It is difficult to say whether *Tagetes patula* might out-compete endemic species, but since these mainly belong to undisturbed areas the threat might not be too serious, at least not yet. Species living in disturbed areas are, however, commonly regarded as rather hardy plants, and may therefore compete better than vulnerable endemic species, not used to competition. The fact that *T. patula* has characteristics valued and developed by horticulturalists probably makes the species a good competitor when conditions allow.

Bidens prestinaria was found in both associations, and appears to be indifferent when it comes to conditions separating “Association 1” and “Association 2” (light intensity, humidity). Observations revealed that it often co-occurs with *Tagetes patula* in open landscapes, and in such situations *B. prestinaria* tends to dominate. In the situation of co-occurrence *B. prestinaria* might create the necessary shade regime for *T. patula*.

4.3 Do the ornamental species of Asteraceae have life history traits that give potential for invasiveness?

Due to the fact that we discovered that *Zinnia elegans* to a very little extent had escaped, this part of the discussion will focus on *Tagetes patula*. *Bidens prestinaria* will be considered for comparison.

Seed production

Among the factors that contribute to the apparent success of *Tagetes patula* as invader in BGNRS, might its ability to produce relatively large numbers of seeds be important. It has been suggested that the size of seeds represents a compromise between the requirement for dispersal (which would favour small seeds) and the requirement for seedling establishment (which would favour large seeds)(Hailu Shiferaw *et al.* 2004). For plants like *T. patula*, occurring in more or less disturbed sites where open ground is created relatively frequently, a large number of small seeds might be more important than large seeds with elaborate dispersal mechanisms. The relatively small size of *T. patula* diaspores facilitates burial because they might easily filter into cracks or small openings that often are created in disturbed soil. The cypselas of *T. patula* have no distinct adaptations for being dispersed by either wind or animals, but they are obviously spread by cattle faeces (endozoochory, cf. Stiles 1992). Endozoochory offers three advantages in that (1) the seeds are dispersed with the faeces at some distance from the parent plant, (2) seeds that pass through the gut of animals are exposed to treatments which may facilitate germination, and (3) the faeces itself may act as fertilizer in the initial, most critical stage of establishment of its seedlings (Hailu Shiferaw *et al.* 2004). However, *Tagetes patula* is probably mainly eaten by accident by domestic animals, apparently trying to avoid it, in search for something else. In addition the cypselas carry a pappus of short hairs that give some possibility for ectozoochory, including dispersal by road construction machines and people's clothes.

In comparison to *Tagetes patula*, *Bidens prestinaria* produced approximately twice as many diaspores per plot. This implies that *B. prestinaria* might have a better dispersal potential than *Tagetes patula*. In addition, *B. prestinaria* has distinct hooks on the cypsela, obviously adapted to more efficient ectozoochory.

Germination

The germination experiment revealed that *Tagetes patula* germinated rapidly, independent on whether the seeds were covered by soil or not. *Zinnia elegans* had a delayed germination compared to *T. patula,* germination also here independent on soil cover. The low total germination rate of *Zinnia elegans,* compared to *Tagetes patula,* might be due to the fact that *Z. elegans* has heavier and thicker cypsela walls than *Tagetes patula.* The fact that a very high fraction of *Tagetes patula* seeds germinated, and the rapid germination and growth, makes *T. patula* a better competitor than the species to which it is here compared.

Bidens prestinaria had a very low germination rate and frequency compared to both the introduced ornamentals. The experiment at the University of Oslo may not have met the demands of the indigenous *B. prestinaria*. It is for example possible that the species may require fire to start germination. Digestion enzymes in cattle might further enhance germination. The seeds may also need a longer period of dormancy.

Flower production in grazed or trampled plants

Species living in disturbed areas where animals graze and trample need some kind of compensatory mechanisms to overcome the loss of mass and meristems. Therefore, it was not surprising that both *Tagetes patula* and *Bidens prestinaria* obtained stimulated growth of side-shoots when the main stem was eaten or trampled. *Tagetes patula* contains chemical substances which grazing animals tend to avoid, and the species is therefore not eaten as much as for example *Bidens prestinaria*. *Tagetes patula* is, however, often trampled upon or broken when animals are trying to reach other species to eat.

Bidens prestinaria produced a significantly higher capitula number in grazed specimens compared to ungrazed specimens. *Tagetes patula* also showed this trend, although the difference was not significant. The dispersal potential is in this way increased, and therefore it is likely that above ground cutting will not be a good method to control the plants. The ability of *Tagetes patula* to regenerate both from seeds and through sprouting from damaged plants makes it a strong competitor, and most likely a successful invader.

Soil seed bank

The density of viable seeds recovered from soil samples collected at the study site indicates that *Tagetes patula* accumulates seed reserves in the soil. Relatively small seeds are characteristic of species which have persistent seed banks in the soil (Thompson and Grime, 1979; Demel, 1998). These reserves serve as one way of regeneration/invasion ensuring recruitment of seedlings in the event of disturbance. Studies conducted in Ethiopia have shown that many, particularly early successional plant species, possess numerous long-lived soil seed banks, which contribute to their perpetuation after disturbance (Demel 1998, Hailu Shiferaw *et al.* 2004). The longevity of the *T. patula* seed bank is, however, not known. Also *Bidens prestinaria* had viable seeds germinating in the soil seed banks.

The different areas where soil seed bank samples were taken, were categorized into three groups. Most *Tagetes patula* seedlings germinated from soil banks from “Site 1”- the *Tagetes* dominated woodland. Surprisingly most *Bidens prestinaria* seedlings also germinated in soil from this site. This might be due to the fact that this species is widespread and has been in the area “always”. Seedlings of the species were also observed in soil from “Site 2” -*Tagetes* and *Bidens* dominated hill and “Site 3” - *Bidens* dominated field.

In conclusion, the introduced *Tagetes patula* is a typical r-strategic plant species (Grime, 1979). It produces a large number of relatively small diaspores, has a seed bank, has a rapid growth rate, and grazing/trampling stimulates growth of side shoots. It is well known that most invasive/weedy plants share these characters. On the other introduced species, *Zinnia elegans,* less information has been collected.

4.4 Could fire facilitate or control the invasion of natural vegetation by *Tagetes patula* and *Zinnia elegans*?

Mechanical scarification and sulphuric acid treatments may be used to simulate fire, and are sometimes preferred to alternative treatments of hot water or dry heat treatments (Cavanagh 1987). The dry heat treatment was, however, chosen because of the available equipment at the University of Oslo, and for easier comparison with studies done on other Ethiopian plant species (Menassie Gashaw and Michelsen 2002).

Results from the fire simulation experiment revealed that 78% of *Tagetes patula* seeds in the study germinated from the control, while germination was lower, but stayed rather high for seeds treated with different dry heat treatments (except the highest temperature impact, were all the seeds died before germination). This suggests that the seeds to a certain degree will withstand fire, unless the heat becomes excessive.

Zinnia elegans showed a higher germination frequency than *T. patula* in all treatments. This suggests that *Z. elegans* seeds have a higher fire resistance than *T. patula*. This might be connected to the fact that *Z. elegans* has the thickest cypsela wall of the three study species. Even though some seeds treated at the highest temperature impact survived, the germination rate was significantly lower compared to the other treatments.

Bidens prestinaria showed no sign of being affected by the different heat treatments, as the difference between the control and the harshest treatment was more or less the same. Being and indigenous species *B. prestinaria* might have been adapted to natural fire regimes through evolution.

Most likely the annual fires existing in Ethiopia, will not be able to control invading species as *Tagetes patula* and *Zinnia elegans*, at least not when the temperatures are low (when there is little fuel). If fire should be used as a control method, temperatures/time regimes similar to the harshest experimental treatment might be necessary to control invasion.

5. Conclusion

The introduced and escaped species, *Zinnia elegans*, is so far not considered to be a threat to the natural landscapes in Western Ethiopia. *Tagetes patula*, however, has several characters that might facilitate invasiveness. It produces a large number of relatively small diaspores, has a seed bank, has a rapid growth rate, and grazing/trampling stimulates growth of side shoots. It is well known that most invasive plants turning weedy, share these characters.

One way to control the growth of *Tagetes patula* might be to pull up the plants or cut the plants near the ground, another way could be to remove the buds before the seeds are mature. All methods would have to be repeated over several years because of the soil seed banks which provide germination under favourable conditions.

People think of *Tagetes patula* as an ornamental species, and they like it. They are not aware of the potential invasiveness of the species. One recommendation will therefore be to inform people about the species characters, and what will happen if its growth increases exponentially. When people become aware of the situation, it will be easier to propose possible management methods.

A way to increase people's interest in *T. patula* is to inform about the economical importance the species has elsewhere. The species is used both as a dye, and as a seasoning in other countries, and this information could make the species more attractive to harvest. Introduction of techniques for collection and for extraction of essential oils could be advantageous in regard to both control and economic development in the country.

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Appendix A

Species in the species association analysis

FAMILY	SPECIES	Coll. Nu.
ACANTHACEAE	?	1572
ACANTHACEAE	*Hypoestes forskaolii* (Vahl.) R. Br.	1545
ACANTHACEAE	*Justicia ladanoides* Lam.	1521
ACANTHACEAE	?	1510
ACANTHACEAE	*Thunbergia alata* Bojer	1506
ACANTHACEAE	*?*	1475
ACANTHACEAE	*Hygrophila attriculata* (Schumach.) Heine	1477
ANACARDIACEAE	*Rhus glutinosa* A. Rich var. *glutinosa*	1515
ANACARDIACEAE	*Lannea fruticosa* (Hochst. Ex A. Rich.) Engl.	1548
ANNONACEAE	*Annona senegalensis* Pers.	1518
APIACEAE	*Centella asiatica* (L.) Urban in Mart.	1453
APIACEAE	*Diplolophium africanum* Turcz.	1528
APIACEAE	*Oreoschimperella verrucosa* (A. Rich.) Rauschert	1509
ASCLEPIADACEAE	*?*	1571
ASTERACEAE	*Tagetes patula* L.	1277
ASTERACEAE	*Bidens prestinaria (Sch. Bip.) Cuf.*	1349
ASTERACEAE	*Crassochephalum rubens* (Juss ex. Jacq.) S. Moore	1444
ASTERACEAE	*Galinsoga quadriradiata* Ruiz & Pavon	1449
ASTERACEAE	*Acmella caulirhiza* Del.	1470
ASTERACEAE	*Ageratum conyzoides* L.	1481
ASTERACEAE	*Galinsoga parviflora* Car.	1482
ASTERACEAE	*Bidens biternata* (Lour.) Merr. & Sherff.	1486
ASTERACEAE	*Bidens prestinaria (Sch. Bip.) Cuf.*	1487
ASTERACEAE	*Bidens pilosa* L.	1575
ASTERACEAE	*Dicrocephala integrifolia* (L. F.) O. Kuntze	1490
ASTERACEAE	*Lactuca inermis* Forssk.	1501
ASTERACEAE	*?*	1513
ASTERACEAE	*Guizotia schimperi* Sch. Bip. Ex Walp.	1525

ASTERACEAE	*Bidens biternata* (Lour.) Merr. & Sherff.	1534
ASTERACEAE	*Bidens prestinaria* (Sch. Bip.) Cuf.	1551
ASTERACEAE	*Ageratum conyzoides* L.	1552
ASTERACEAE	*Ethulia gracilis* Del.	1553
ASTERACEAE	*Vernonia theophrastifolia* Schweinf. Ex Oliv. & Hiern	1560
BIGNONIACEAE	*Stereospermum kunthianum* Cham.	1544
CAMPANULACEAE	*Lobelia inconspicua* A. Rich	1472
CELASTRACEAE	*Maytenus senegalensis* (Lam.) Exell.	1543
COMBRETACEAE	*Anogeissus leiocarpa* (A.DC.)Guill. & Perr.	1579
COMBRETACEAE	*Combretum collinum* Fresen	1580
COMMELINACEAE	*Commelina latifolia* Rich.	1483a)
COMMELINACEAE	*Commelina imberis* Ehrens. Ex. Hassk	1483b)
COMMELINACEAE	*Commelina subulata* Roth.	1456
COMMELINACEAE	*Aneilema hirtum* A. Rich	1448
CUCURBITACEAE	*Momordica foetida* Schumach.	1566
CUCURBITACEAE	*Cucumis prophetarum* L.	1561
CYPERACEAE	*Cyperus flavescence*	1462
CYPERACEAE	*Cyperus metzii*	1463
CYPERACEAE	*Fimbristylis dichotoma*	1465
CYPERACEAE	*Burbostylia hispidula*	1500
CYPERACEAE	*Cyperus sesquiflorus*	1507
CYPERACEAE	*Cyperus divulsus*	1479
DIOSCOREACEAE	*Dioscorea bulbifera* L.	1577
EUPHORBIACEAE	*Phyllanthus pseudoniruri* Muell. Arg.	1450
EUPHORBIACEAE	*Euphorbia hirta* L.	1497
EUPHORBIACEAE	*Flueggia virosa* (Willd.) Voigt	1520
EUPHORBIACEAE	*Acalypha villicaulis* A. Rich.	1547
EUPHORBIACEAE	*Croton macrostachyus* Del.	1578
FABACEAE	*Crotalaria ononoides* Benth.	1494
FABACEAE	*Acacia hecatophylla* A. Rich	1563
FABACEAE	*Senna obtusifolia* (L.) Irwin & Barneby	1564
FABACEAE	*?*	1555
FABACEAE	*Indigofera spicata* Forssk.	1541
FABACEAE	*Alysicarpus rugosus* (Willd.) DC.	1531
FABACEAE	*Rhynchosia nyasica* Bak.	1522

FABACEAE	*Acacia polystachya* A. Cunn ex. Benth.	1523
FABACEAE	*Entada africana* Guill. & perr.	1492
FABACEAE	*Albizia malacophylla* (A. Rich.) Walp.	1574
FABACEAE	*Indigofera spicata* Forssk.	1467
FABACEAE	*Trifolium rueppellianum* Fresen	1445
FLACOURTIACEAE	*Flacourtia indica* (Burm. F.) Merr.	1573
HYPOXIDACEAE	*Hypoxia angustifolia*	1504
LAMIACEAE	*Leucus martinicensis* (Jacq.) R. Br.	1446a)
LAMIACEAE	*Leonotis osymifolia (Burm. F.) Iwarsson*	1446b)
LAMIACEAE	*Plectranthus*	1447
LAMIACEAE	*Plectranthus*	1480
LAMIACEAE	*Ocimum trichodon* Baker ex. Gûrke	1530
LAMIACEAE	*Ocimum trichodon* Baker ex. Gûrke	1537
LAMIACEAE	*Leonotis osymifolia* (Burm. F.) Iwarsson	1559
MALVACEAE	*Sida urens* L.	1491
MALVACEAE	*Sida alba* L.	1554
MALVACEAE	*Sida ovata* Forssk.	1469
MORACEAE	*?*	1557
MORACEAE	*Ficus sycomorus* L.	1517
MYRTACEAE	*Syzygium guineense* (Willd.) DC.	1581
OLEACEAE	*Jasminum grandiflorum* L. Subsp. *Floribundum* (R. Br. Ex. Fresen.) P. S. Green.	1524
OLEANDRACEAE	*Nephrolepis undulata*	1512
ORCHIDACEAE	*Nervilia kotschyi var. purpurata* (Rchb. F. & Sond) B. Pettersson.	1511
OROBANCHACEAE	*Orobanche minor* Smith.	1505
POACEAE	*Eleusine africana* Kenn. -O`Byrne	1484
POACEAE	*Setaria pumila* (Poir.) Roem. & Schult	1452
POACEAE	*Panicum pusillum* Hook. F.	1454
POACEAE	*Digitaria ternata* (A. Rich.) Stapf.	1455
POACEAE	*Brachiaria brizantha* (A. Rich) Stapf.	1457
POACEAE	*Sporobolus piliferus* (Trin.) Kunth	1460a)
POACEAE	*Sporobolus africanus* (Poir.) Robyns & Tournay	1460b)
POACEAE	*Brachiaria semiundulata* (A. Rich) Stapf.	1466
POACEAE	*Paspalum scrobiculatum* L.	1468
POACEAE	*Oplismenus burmannii (Retz.) P. Beaur.*	1476

POACEAE	*Aira caryophyllea L.*	1478b)
POACEAE	*Arthraxon micans* (Nees) Hochst.	1489
POACEAE	*Panicum atrosanguineum* A. Rich	1495
POACEAE	*Eragrostis schweinfurthii* Chiov.	1496a)
POACEAE	*Sporobolus festivus* Hochst. Ex A. Rich	1496b)
POACEAE	*Sporobolus piliferus* (Trin.) Kunth	1498
POACEAE	*Chloris pycnothrix* Trin.	1499
POACEAE	*Hackelochloa granularis* (L.) O. Kuntze	1502
POACEAE	*Aira caryophyllea* L.	1503
POACEAE	*Pennisetum polystachion* (L.) Schult.	1527
POACEAE	*Sporobolus panicoides* A. Rich	1529
POACEAE	*Cynodon dactylon* (L.) Pers.	1542
POACEAE	*Oplismenus burmannii* (Retz.) P. Beaur.	1538
POACEAE	*Echinocloa colona* (L.) Link	1539
POACEAE	*Digitaria ternata* (A. Rich.) Stapf.	1546
POACEAE	*Dactyloctenum aegypticum* Beauv.	1556
POACEAE	*Pennisetum petiolare* (Hochst.) Chiov.	1569
POACEAE	*Schizachyrium brevifolium* (Sw.) Bûse	1565
POACEAE	*Schizachyrium brevifolium* (Sw.) Bûse	1565
POLYGALACEAE	*Polygala persicariifolia* DC.	1488
POLYGONACEAE	*Persicaria nepalensis* (Meissn.) H. Gross	1458
PRIMULACEAE	*Asterolinon adoense* O. Kunze	1473
PRIMULACEAE	*Asterolinon adoense* O. Kunze	1464
RANUNCULACEAE	*Clematis hirsuta* Guill. & Perr.	1536
RHAMNACEAE	*Ziziphus abyssinica* Hochst. Ex A. Rich.	1582
RUBIACEAE	*Spermacose chaetocephala* DC.	1493
RUBIACEAE	*?*	1562
RUBIACEAE	*Spermacose sphaerostigma* (A. Rich.) Vatke	1451
RUBIACEAE	*Spermacose sphaerostigma* (A. Rich.) Vatke	1461
RUBIACEAE	*Kohautia tenuis* (S. Bowd.) Mabb	1474
SANTALACEAE	*Osyris quadripertita* Decn.	1519
SCROPHULARIACEAE	*Lindernia nummulariifolia* (D. Don) Wettstein	1471
SELAGINELLACEAE	*Selaginella kraussiana* (Kze.) A. Br.	1485
SOLANACEAE	*Solanum incanum* L.	1583
TILIACEAE	*Triumfetta rhomboidea* Jacq.	1576

TILIACEAE	*Triumfetta rhomboidea* Jacq.		1550
TILIACEAE	*Triumfetta pilosa* Roth.		1532
TILIACEAE	*Grewia ferruginea* Hochst. Ex. A. Rich		1508
URTICACEAE	*?*		1540
VITACEAE	*Cyphostemma adenocaule* (Steud. Ex A. Rich.) Des. ex Wild. & Drum.		1570
VITACEAE	*?*		1535
VITACEAE	*Cyphostemma cyphopetalum* (Fresen.) Des. ex Wild & Drum.		1459
Undetermined	?		1558
Undetermined	?		1533
Undetermined	?		1526
Families;	40	Species; 142	